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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. No. 91

ON THE SIGNIFICANCE OF THE REACTION TO SHADING IN CHITON

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I. The objections which certain writers have urged against the theory of phototropism in animals, as originally propounded by Loeb and so brilliantly developed and supported by his later quantitative researches, are of considerable variety: thus upon the one hand we note that in different cases the fact of photic orientation is either denied, or its method is stated to be indirect, or it is dismissed as a "laboratory product" (which is quite beside the point); while upon the other hand we find, for cases where the orientation-process itself is unmistakably clear, that exception is more specifically taken to the conception of direct photic excitation, which the tropism theory advances. It is with an exception of the latter category that we propose to deal in this paper.

Developing an idea originally made prominent by Jennings, Mast ('11, '14) has been the most active defender of the proposition that the orienting stimulus in photic responses can in general be traced to the "time rate of change of intensity" of the incident light upon photoreceptors. This view of the method of excitation in phototropic movements obviously demands, among other things, at least these two

preliminary conditions: (1) A change of the incident light intensity must actually be shown to take place with an effective "time rate;" and (2) the known response to change in light intensity, when such form of reaction takes place, must be of a character appropriate to produce orientation of the kind (negative or positive) actually exhibited by the particular animal concerned. The latter condition is, as a matter of fact, very generally realized; thus Mast remarks ('11, p. 265):

> In many of the lower forms [how low is not indicated] orientation results from responses to change in light intensity. When these forms are negative they respond only to an increase of intensity, and when they are positive only to a decrease.

The behavior of some very slowly moving animals in an illuminated field gives valuable information relative to the first of the necessary conditions to which we have referred, and there are a few animals in which the second of these conditions can be subjected to examination. We are concerned in this communication with the photic behavior of an animal which seems especially valuable in relation to both of these conditions, but more particularly to the latter, since its response to change in light intensity ("differential sensitivity") is always of a character consistent with the sense in which photic orientation takes place. This animal is Chiton tuberculatus Linn., the common intertidal chiton of the Bermudas. The behavior of this 'primitive' mollusk has not previously received attention, presumably because of the reputation for persistent sluggishness which the Placophora in general enjoy; nevertheless, their activities prove, upon careful scrutiny, to exhibit a number of exceptionally interesting features.[1]

II. Young individuals of this species of chiton are almost always located under loosely piled, flat stones, in relatively dark surroundings, at about the upper limit of the tides. This is particularly true of specimens less than 2 cm. long; when still younger, less than 1 cm. long, the chitons are commonly found beneath low-water level. Somewhat older animals are also found in this type of habitat but after they attain a length of 6 cm. they commonly frequent more exposed situations. Chitons of the largest size (8 to 9 cm.) are most usually found freely exposed upon the intertidal zone of the shore rocks although they also inhabit crevices, semiconcealed depressions, the under sur-

[1] For an account of the behavior of this animal and an analysis of its reactions, we may refer to a forthcoming report by Arey and Crozier on "The sensory responses of Chiton" (cf. Arey, '18).

face of large flat rocks and other more or less dark places. Characteristically, however, the young chitons are found in dark situations, while the oldest ones live in the light.[2] When the photic responses of the young chitons and of the old, taken from these respectively characteristic habitats, are studied in comparison, it is found that the smallest chitons are uniformly photonegative, the larger ones photopositive. A miscellaneous collection of individuals may be caused to separate into two general size-groups by means of their respective reactions to sunlight falling obliquely upon them. They become oriented in a diagrammatic manner. It is necessary to study their reactions when immersed in seawater, for although the smallest specimens (less than 2 cm. long) move about actively upon a moist surface, the older ones do not creep freely when in air.

This difference in the behavior of the chitons of large and of small size respectively is clean-cut and thoroughly consistent when the extremes of size are compared. When animals of intermediate size, 5 to 7 cm. long, were studied it became clear that the sense in which orientation occurred depended upon the intensity of the light employed. An experiment illustrating this behavior may be cited: Owing to the relatively large size of the animals and to the slowness with which they move, it has been convenient, as well as necessary, to give careful attention to the responses of isolated single individuals.

Chitons 123.5, 123.7. Obtained from a deep crevice in the shore rock on the north side of Long Island. Placed in a dark container and protected from light until tested.

9.00 a.m. Placed in sea water in a shallow, rectangular glass vessel illuminated by diffuse light from a north window; each animal so placed that its long axis was transverse to the light.

9.07 a.m. Both chitons oriented *toward* the window, *toward* which each crept for 12 cm. in a straight line.

9.15 a.m. Animals placed as before at the center of the glass vessel, one headed into the light, the other transverse to it. Direct sunlight reflected horizontally upon the chitons.

9.16 a.m. Both began orienting *away* from the light; crept *away* from the light as far as possible.

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[2] The smaller chitons, consistently found under stones, are all less than two years old. With increasing age there is an increasing tendency to frequent illuminated areas; this correlation is not mathematically exact but is clear and unmistakable, so that individuals eight years old or older are almost always found in the light. The evidence supporting these statements regarding age cannot be given here, but will be found in a subsequent paper by one of the present writers (W. J. C.) treating of the bionomics of chiton.

9.25 a.m. Tested again, this time with light reflected from the north sky. Both animals diagrammatically positive.

10 a.m. to 12 m. Four further alternations of weak and strong light gave results completely consistent with the preceding.

Chiton 123.5 was a male 6.6 cm. long, no. 123.7 a female[3], 6.7 cm. long.

All that we wish to show at this point is that small (young) chitons are photonegative, the largest ones characteristically photopositive, and those of intermediate size positive or negative according to the intensity of illumination. The cause of this change in behavior is not important for our further conclusions in this paper, although, as we shall subsequently indicate, it has an important significance for the method of origin of some conspicuous bionomic correlations which this chiton displays.

III. Chiton tuberculatus is also reactive to shading. It is in fact remarkably sensitive to a very small, but sudden, decrease in light intensity. When a chiton is creeping quietly in moderately bright sunlight, the shadow produced by a fly, six feet distant, passing between it and the sun, will cause the animal's girdle to be firmly applied to the substratum; after a half minute or perhaps less the chiton continues its creeping toward the light, while in other instances small, momentary shadows produce merely a local depression of the girdle. *If the same individual chiton be caused to orient and to make locomotor progress away from a stronger source of light, it gives during this process reactions to shading which are of measurably greater amplitude.* Chitons of all ages and from every variety of habitat are consistent in their behavior toward shading. The reaction to suddenly decreased illumination is, to shadows of small area, local in character, and at its maximum consists (when the animal is creeping on a solid substratum) in ventral-ward contractions of the girdle, cessation of locomotion and contraction of the musculature of the foot and body generally; the magnitude of these movements depends largely upon the original intensity of the light and the amount of the chiton's surface which may be shaded. The response to shading is here essentially of that prompt, precise, local and predictable character which one finds exhibited in other mollusks and in various echinoderms and has led to its being loosely and somewhat gratuitously denominated a "reflex" to shading.

The ventral surface of chiton is also sensitive to shading. In this general distribution of differential sensitivity over its surface, chiton differs considerably from such photopositive gastropods as Conus and

[3] Cf. Crozier ('18).

certain nudibranchs, for example. The response to shading of the ventral surface comprises, in cases where the response is maximal, a complete rolling up of the shell and contractive movements upon the head, foot, girdle and ctenidia. Under certain conditions this response on the part of Chitons fully extended but resting on their dorsal surface, is exceedingly delicate, both to shading, to touch and to other forms of excitation. Never under any circumstances does chiton respond to an increase in illumination, however, whether the dorsal or the ventral aspect of the animal is involved in the experiment, except as noted in the following:

In older chitons the periphery of the girdle is sensitive, and reactive, to increase of light intensity, provided the final intensity be that of brilliant sunlight. The response is that already described, a depression of the girdle to the substratum. It is much less vigorous, much less complete, and more slowly carried out, than is a response to an equivalent shading. So far as it goes, this response completes the cycle of qualitative proof that changes of light intensity, as such, are of no concern in phototropic movements—because their sense is inconsistent with that in which orientation takes place. The girdle reaction to light suddenly made brighter is present under conditions in which the chiton is photopositive. Moreover, the girdle is not involved in photoreception leading to orientation, since this process remains the same when the girdle has been removed (by amputation).

IV. Thus Chiton tuberculatus is in its younger stages photonegative, in its later years of life characteristically photopositive, toward ordinary sunlight. Individuals of all ages, independently of the type of habitat from which they may be taken and regardless of the sense in which they are found to be oriented by light of a given intensity, indicate by motor responses of a uniform character that they are negatively reactive to sudden diminution in light intensity. They do not react in this way to a sudden increase in light intensity. It may be emphasized here, as in the case of certain pedate holothurians (Crozier, '14, '15), that the simultaneous presence of *photonegative* orientation and a precise *negative* response to shading, without any response (of the part concerned in orienting reactions) to increased illumination, is thoroughly inconsistent with the idea that photonegative orientation is bought about by a stimulation induced through any change in light intensity, as such.

The behavior of chiton is especially significant, however, since the older individuals are to various degrees (and in many cases very strongly) photopositive, though still fully reactive to shading; and since in any given chiton of medium size photopositive or photonega-

tive orientation may be brought about at will by appropriately controlling the acting luminous intensity, yet without impairing the constant sense of its "differential sensitivity." We are, then, entirely at liberty to believe, and indeed forced to expect, that in other animals also photopositive orientation may have no organic connection of any sort with such sensitivity to shading as they may exhibit.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. NO. 310

REACTIONS OF FROGS TO HEAT AND COLD

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INTRODUCTION

The purpose of these experiments was to determine the behavior of the leopard frog, Rana pipiens Schreber, in water of different temperatures.

That the body temperature of the frog and of other cold-blooded vertebrates is very little, if at all, above that of their surroundings seems to be a well attested fact (Milne Edwards, '68; Rogers and Lewis, '16). Knauthe ('91) and Müller-Erzbach ('91) have studied the effects of low temperatures on the frog dealing more, however, with its resistance to cold than with its behavior. Knauthe found that frogs could survive a twelve hours' exposure to a temperature ranging from $-1°$ C. to $-5°$ C., during which their body temperature sank from $-0.2°$ C. to $-0.8°$ C. Most of the frogs failed to recover when the body temperature was reduced to $-0.9°$ C. Müller-Erzbach froze frogs in water and exposed them to a temperature of from $-4°$ C. to $-6°$ C. for several hours. They afterwards revived.

Maurel et Lagriffe ('00) decided that the frog cannot survive a temperature of $-5°$ C. but may survive a temperature of 0° C. to $-3°$ C. These investigators studied the effect on the frog of temperatures from $-4°$ C. to 41° C., pointing out a certain parallelism between the effects of abnormal cold and abnormal heat.

Torelle ('03) described the behavior of the frog at various temperatures. She found that the positive phototactic response in a dry box was much accelerated by raising the temperature to 25° C. Between 25° C. and 30° C. the frog grew restless. Above 30° C. it no longer responded to light. At a temperature of about 8°C. the frog became sluggish and was negatively phototactic. When placed in a jar of

water at this temperature, the frog swam down to the bottom where it crawled about. It did the same when either the upper or lower two-thirds of the jar were darkened. The frog appeared stereotropic in water at temperatures between 4° C. and 10° C., trying to crawl under stones or get between them and the jar.

Babák ('13) also studied the stimulation of frogs by changes of temperature. A frog from which the forebrain had been removed increased the rate of its pharyngeal respiratory movements when a warm thermaesthesiometer was held at about 1 mm. from its skin and decreased the rate when cold was similarly applied.

Judging from the results thus far cited it seems that the frog can withstand a temperature at least as low as −3° C. It swims down and is sluggish in water at temperatures below 10° C., and it is especially active at temperatures from 25° C. to 30° C.

The present study of temperature reactions was suggested by G. H. Parker and carried on under his direction. Two main points were considered:

1. The percentage of time that a frog will spend at the surface of water at different temperatures in trials of fifty minutes duration; and 2. The number of times that the frog will go from the surface to the bottom and vice versa during these trials.

METHODS

In making observations on the frogs I used the following apparatus. Two glass jars 32 cm. high by 24 cm. in diameter, filled with water to a depth of 22 cm. were placed side by side on a small table. Thick wads of paper were kept under the table legs to exclude any disturbing vibrations that might be transmitted through the floor. Each jar was surrounded by a screen that cut off all light except that which came from in front. The jars were illuminated by diffuse light from the laboratory windows. The observer sat in front of the jars at a distance of 4 or 5 meters and the utmost care was taken not to disturb the frogs during an experiment by movements or noises. The screening of the jars was so arranged that the movements of a frog in one jar could not be seen by a frog in the other jar.

In making the tests, the jars were partly filled with water at the desired temperature and the frogs put in. The main stock of frogs was kept in a tank in the basement of the laboratory where it was light, but those to be tested were generally kept in jars in the laboratory for a day or so before the tests were made.

Each experiment began with a preliminary twenty-five minutes during which the frog was allowed to become accustomed to the new situation and to recover from the disturbance of having been handled. After this period had passed, record of the frog's behavior for the next fifty minutes was made, the whole proceeding thus requiring one hour and a quarter. Usually two frogs were tested at once, one in each of the two jars previously mentioned.

A graphic record was kept of the behavior of each frog. The temperature of the water at the beginning and end of each experiment was read to 0.1° C. from a calibrated thermometer graduated to degrees. The temperature of the water during the period of an experiment, one hour and a quarter, varied at most 5° C., a common change being about 2°C. The average for the fifty-minute trial was estimated by adding to or subtracting from the final temperature one-third the difference between the initial and final temperatures.

Ten records of this sort, obtained from ten different frogs, were made for every 5° C. from 0° C. to 35° C. Thus, each record of the first set has an average trial temperature between 0° C. and 5° C.; while those of the second set have average temperatures between 5° C. and 10° C. The first records were made on February 21 and the last on May 12. This time of year is usually not the most favorable one for work on frogs as many of them die, but tests were made on only such animals as were in good condition.

In discussing the results of these experiments, the general behavior of the frogs at the various temperatures may be taken up first, after which the interpretation of their behavior will be considered.

Temperatures from 0°C. to 5°C. When first placed in the jar, a frog generally swam down to the bottom immediately. This was usual at all temperatures because the animal was disturbed by handling. In water at temperatures between 0° C. and 5° C., the frog then commonly tried to regain the surface by a series of hard kicks, becoming more feeble as the animal grew stiff. It then settled down to the bottom by gravity. This was quite distinct from swimming down. It remained at the bottom practically all the time during the preliminary twenty-five minutes and the fifty minutes of the real trial. It was very sluggish, occasionally crawling around the bottom, moving the hind legs alternately, or making a few kicks toward the surface. If it reached the top of the water it usually sank down again very soon, frequently into an erect position with hind feet resting on the botton. In sinking, it sometimes fell over on its back and then righted itself gradually.

When quiet, it was crouched or stretched out on the bottom of the jar or erect. The frog was often apparently benumbed when taken from the jar, and would lie quietly on its back in one's hand. Maurel et Lagriffe ('00) note loss of the sense of equilibrium between 6°C. and 7°C. When put in water at room temperature, the frog became active in a minute or so.

Among the frogs tested one marked exception to this general description was noted. This frog floated 68 per cent of the time in water at a temperature of 1.5°C. It went from the top to the bottom of the jar forty times during the trial, swimming down rather than sinking. This frog behaved so entirely differently from the others that its record was omitted in plotting the curves to be discussed later.

The average temperature of the ten trials was 2.8°C. The time spent at the surface varied from none (eight cases) to four per cent, with an average of 0.5 per cent. The number of times the frog went between surface and bottom during the fifty-minute trial varied from none (five cases) to twelve, with an average of 2.2 times.

Temperatures from 5°C. to 10°C. Frogs in water at these temperatures were distinctly more active than in water from 0°C. to 5°C., though they were still somewhat sluggish. They generally swam rather than sank down and spent most of the time crouched on the bottom or crawling about there.

The average temperature of the ten trials was 7.1°C., the propor ion of time at the surface varied from none (three cases) to fifteen per cent, with an average of 4.2 per cent. The number of times the frog went up or down varied from none (one case) to forty-six, averaging 18.3 times.

Temperatures from 10°C. to 15°C. At these temperatures the activity of the frogs was much greater than in the preceding sets of trials. The animals swam up and down very freely and did not sink. They frequently floated at the surface, seldom, however, for more than a minute at a time.

The average temperature in the ten trials was 11.9°C. The frogs were at the surface from 3 to 48 per cent of the time, averaging 16.4 per cent. They swam up or down from two to one hundred and thirty-seven times during the fifty minutes, with an average of 55.4 times.

Temperatures from 15°C. to 20°C. At these temperatures the frogs were slightly more active than in the preceding set of experiments and floated more. In two cases the animals floated quietly nearly the whole fifty minutes of the trial. When they floated thus, they often had their hind legs spread out on the surface of the water; when they came up for a short time the hind legs generally hung down.

The average temperature of the ten trials was 17.6°C. The time spent at the surface varied from 1 per cent to 100 per cent, with an average of 37.9 per cent. The number of times the frogs went up or down varied from one to two hundred and sixteen, averaging 64.2 times.

Temperatures from 20°C. to 25°C. At these temperatures the behavior of the frogs was decidedly variable, but they swam up and down less than at temperatures slightly higher or lower. Some frogs floated quietly most of the time; others stayed crouched or occasionally erect at the bottom; others swam up and down repeatedly. In one instance the frog sank, somewhat as in cold water, instead of swimming down, taking at least fifteen seconds to settle from the top to the bottom.

The average temperature was 22.3°C. The frogs spent from 1 per cent to all of the time at the surface, averaging 52.6 per cent, and went up or down from none to one hundred and four times, averaging 26.7 times.

Temperatures from 25°C. to 30°C. The results of these trials were similar to those between 20°C. and 25°C. except that the average activity of the frogs was considerably increased in this set. However, there were some cases of quiet floating. The frogs showed some effects of the slightly unusual heat. Their movements tended to be rapid and jerky. One active animal usually swam down but sometimes sank; another fell over on its back at one time. This may have been due to a slight loss of the sense of equilibrium, which Maurel et Lagriffe ('00) found to be conspicuously the case in warmer water (34°C. to 36°C.).

The average temperature was 27.3°C. The proportion of time at the surface varied from 13 to 100 per cent, averaging 52.4 per cent. The number of times the frogs went up or down varied from none to one hundred and thirty-one with an average of 75.9 times.

Temperatures from 30°C. to 35°C. At these temperatures the frogs more clearly showed disturbance from the heat. When first placed in the jar they swam to the bottom as usual, then after a minute or so began swimming up and down rapidly. Sometimes the frog would then sink down, remain quiet at the bottom, give a spasmodic kick up and sink again with body held motionless. One frog did this ten times in eleven minutes during the preliminary twenty-five minutes, but stopped sinking before the period of the final test. Two continued sinking during this period. This sinking was not restricted to high or low temperatures For it was noted once that a frog sank in water at room temperature. However the tendency seemed much more marked in water below 5°C. and above 30°C.

Though decidedly active during part of the preliminary twenty-five minutes, the frogs were relatively quiet during the trial period, floating a good deal and making occasional sudden dives and splashes even when apparently undisturbed. The difference in behavior between the preliminary and the trial periods might be accounted for either by the assumption that the frog had become accustomed to the heat or by the fact that the water had cooled off a little. The temperature usually fell about 4°C. during the one hour and a quarter. The experiments of this set had the largest range of temperature.

The average temperature was 31.9°C. The frogs floated from 42 per cent to all of the time, averaging 57 per cent. The number of times the frogs went up or down varied from none to ninety-five, with an average of 30.6 times.

Temperatures above 35°C. Frogs put in water at a temperature of about 38°C. swam around very vigorously at first, then in a couple of minutes became quiet. They were promptly removed from the jar, motionless, as when taken from water at a temperature below 5°C. Placed in cold water, they revived in a minute or two. One frog was put in water at a temperature of about 42.5°C. It swam about with extreme rapidity and when taken out within about a minute lay on its back apparently dead. After being in water at room temperature about an hour, it swam a little but did not fully recover. Next day it was dead. Temperatures over 35°C. are probably above the physiological limit of normal activity.

DISCUSSION

The preceding descriptions may be summarized by means of curves, figures 1 and 2. In these graphs each curve is drawn from seven points. Each point in curve *B* of both graphs is based on the average of ten records, while the points in *A* and *C* represent the extremes in these records. In both graphs the abscissas represent temperature in degrees centigrade and the ordinates either the percentages of time at the surface (fig. 1) or the number of excursions made by the frogs (fig. 2).

In the percentage of time spent at the upper surface of the water (fig. 1) the lowest point on each of the three curves is not significant in showing a reaction, for below 5°C. the frogs did not swim but merely sank because of their inaction and specific gravity. This was the regular occurrence unless the frogs' throat or lungs were inflated with air.

In swimming down frogs frequently blew out bubbles of air. The average curve, *B*, shows a smooth rise between about 7°C. and 22°C., indicating the frog's tendency to seek deep water at low temperatures. The upper part of this curve is hardly significant. It merely shows that in warmer water the frogs were at the top about half the time, a condition that might well be attributed to chance without reference to temperature.

The curves that represent the maximum, *A*, and the minimum time, *C*, spent at the top indicate by their distance from each other the range

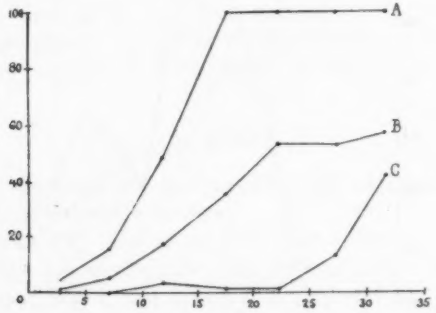

Fig. 1. The percentages of time spent by frogs at the upper surface of the water at temperatures varying between 0° and 35°C. The abscissas represent temperature in degrees centigrade. The ordinates represent in percentages the proportions of the 50-minute intervals spent by the frogs at the top of the water. Curve *A* shows maximum percentages, curve *B* average percentages and curve *C*, minimum percentages of time spent at the top. Each of the seven points in curve *B* represents the average of ten records, while the points in curves *A* and *C* show the extremes of these records.

of variability of the different frogs. This variability is least at the lower end of the curve, where the frogs were largely controlled by gravity, and it then increases rapidly, being greatest between about 17°C. and 22°C., where the temperature might be considered neutral and not strongly stimulating, thus leaving the frog's behavior more dependent upon other factors. At still higher temperatures, variability is somewhat reduced. Averages determined for a range of 5°C. from ten records having results varying as widely as these do are of course likely to be farther from the true averages than in cases where the records obtained agree more closely.

The activity of the frogs as indicated by the number of times they went from the surface to the bottom of the water or vice versa, is shown in figure 2. This is a fair measure of their activity, for the jars were too small to allow much horizontal swimming. The curve for the average

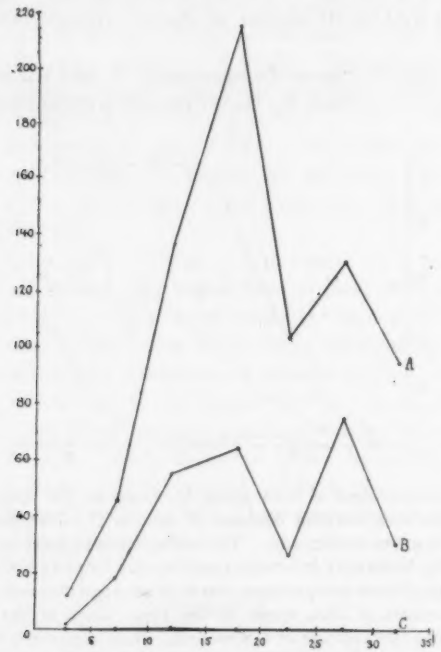

Fig. 2. The activity of frogs at temperatures varying from 0° to 35°C. The abscissas represent temperature in degrees centigrade. The ordinates represent the numbers of times the frogs ascended and descended in the water in each 50-minute interval. Curve *A*, maximum number of excursions; curve *B*, average number of excursions; curve *C*, minimum number of excursions. Each of the seven points in curve *B* represents an average of ten records, while the points in curves *A* and *C* show the extremes of these records.

of the records, *B*, shows a marked increase in activity between about 3°C. and 12°C. It is doubtful whether the rest of the curve is of much significance, as the averages obtained could easily be far from true averages because of the great variation in the records. It is, however, of some interest to compare curve *B* with the observations of Maurel et

Lagriffe ('00). They consider the interval from 20°C. to 25°C. to cover the normal temperature for the frog. This is a region of rather low activity on the curve. From 27°C. to 29°C. and from 11°C. to 15°C., Maurel et Lagriffe note "hyperexcitabilité." In curve *B* about 27.5°C. on one side of normal and about 17.5°C. on the other are points of greatest activity. It should be noted, however, that these are not sharply determined points but merely the averages of ten temperatures between 25°C. and 30°C., and 10°C. and 15°C., respectively. A decrease in activity above 27.5°C. and below 17.5°C. is apparent in the curve, corresponding to Maurel et Lagriffe's "hypo-excitabilité" between 30°C. and 33°C., and 8°C. and 10°C. Curves *A* and *C* together show the great variability of the records on which curve *B* is based and the consequent uncertainty of this curve for the higher temperatures.

SUMMARY

Below 5°C. the frogs became very sluggish and inactive. Gravity caused them to settle to the bottom.

Between about 5°C. and about 20°C. the colder it was the less the frogs were at the top of the water and the less active they were.

Between about 20°C. and 30°C. the frogs did not show any very definite reactions to temperature and their movements were highly variable.

Above 30°C. they showed somewhat decreased activity and a tendency to sink in the water.

Above 35°C. the heat had an injurious effect upon them.

Much more variation in behavior is shown at high than at low temperatures.

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ADRENALIN VASODILATOR MECHANISMS

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From the results of recent work regarding the vasomotor reaction to adrenalin several facts seem to have been established. It has been found that in anaesthetized cats and dogs the arterioles supplying skeletal muscle dilate when small quantities of adrenalin are injected into the circulation and that their reaction changes to constriction when the concentration of adrenalin is sufficiently increased (1), (2). The vessels of the intestinal tract have been found to give the opposite response since they constrict when small, and dilate when large doses are injected (3). It has been shown that there are many parts of the organism, bone (4), skin (5), spleen (3), (6), and possibly kidney (3), (7), the vessels of which show no active dilatation from doses of any strength. It is further conceded by the most recent workers (2), (8),(9) that all blood vessels which are dilated by small quantities of adrenalin lose this reaction, for the time being at least, when separated from central control by cutting their nerves. Dilatation under these circumstances is generally replaced by constriction. The reason for this change in reaction and the whole question of the mechanisms involved are still debated. It has been repeatedly suggested that the conflicting effects of adrenalin in varying concentration are entirely due to its stimulation of neuromuscular junctions of two kinds, one constricting and the other dilating. Those who hold this view believe that vessels which have been recently denervated fail to respond by dilatation to adrenalin because of loss of tone (9), (10).

Work from this laboratory has shown that stimulation of the sympathetic and dorsal root ganglia by adrenalin is sufficient to account for the dilatation (11). Although Gruber has shown that some time after denervation peripheral mechanisms respond in a similar manner, this might be due to loss of sensitivity by the constrictor myoneural junctions. The present research is an investigation of this problem.

METHODS

The methods employed are those of the previous researches described in this Journal, with some modifications and additions. Adrenalin chloride solution (Parke, Davis & Company) was used except in one experiment, in which a more concentrated solution was needed for direct application to ganglia. In this case we used pure adrenalin, made by the same firm. Blood pressure was taken from the carotid artery and injections into the general circulation were made by way of the jugular vein. In order to reduce the constrictor effects of the skin, we eliminated the paw by using a metal cuff open at both ends, a side-tube furnishing connection for the bellows. Both ends of the cuff were made air-tight by packing with a vaseline-cotton or vaseline-paraffin-cotton mixture. In the perfusion experiments, when records were to be taken of one hind limb only we put the cannula into the common iliac artery; when both limb volumes were being recorded we perfused through the abdominal aorta immediately above the bifurcation. The perfusion fluid was allowed to escape through slits in the iliac vein or veins directly into the abdominal cavity since any attempt to lead it away through cannulae from the veins resulted sooner or later in clotting. In some experiments we had difficulty in getting an equal flow of the perfusion solution to the two limbs. Results from these were of course discarded. The difficulty was found to be lessened by tying the internal iliac and the middle sacral arteries. The pressure employed for perfusion varied in different experiments between 10 mm. and 50 mm. Hg., the average being about 20 mm.

In all experiments involving denervation of a limb both the sciatic and femoral nerves were severed. Aseptic precautions were observed in those animals which were to be kept for later use. In none of our experiments did infection of the muscles result. The skin suppurated in a few cases due to post-operative infection, but this seemed in no way to affect the muscle.

In the two experiments (p. 505) in which the changing volume of a limb after denervation was to be continuously recorded as well as its response to periodic doses of adrenalin, we connected the plethysmograph by means of a T-piece to two bellows, one large and one small. The slow changes were recorded on the larger one, while the little one (deflated) was clamped off. When the time came for injection, the clamp was removed from the small bellows tube, the latter bellows being slightly inflated by a small compression of the larger bellows, then the

tube leading to the large bellows was clamped. The small bellows was thus prepared to register a small volume change in the limb. After the injection effects were finished, the clamp on the large bellows tube was removed, the small bellows was deflated by forcing the air into the large bellows and then clamped off. By this method no air was lost during the experiment.

RESULTS

Response after recent denervation. The peripheral effect of adrenalin was compared with the total "gangliar peripheral" effect in fifteen cats

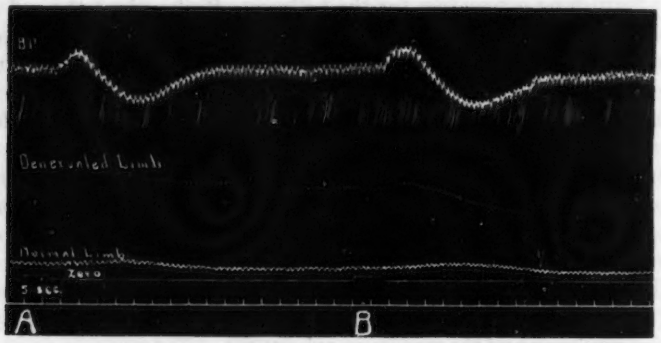

Fig. 1. Small active dilatation (A) of a denervated limb which occurs when 0.2 cc. of adrenalin, 1: 100,000 is given disappears when a slightly larger dose 0.4 cc. of the same solution, (*B*) is injected. Although the bellows for the normal limb was less sensitive, it does show that the maximum dilatation of the normal limb coincides with the maximum fall in blood pressure while the dilatation of the denervated limb does not coincide. Cat, 3.3 kgm. (Reduced one-half)

by studying the volume changes in a denervated limb simultaneously with those in a normal limb (2). The response of the denervated limb was predominantly constriction, although there was a short period of dilatation which usually occurred at the time of the blood pressure rise. Except in a few instances this was undoubtedly a passive effect. In these the dilatation persisted for a short time during the blood pressure fall and came earlier than that in the normal limb (fig. 1, *A*). This dilatation occurred only from small doses of adrenalin, a small increase in the adrenalin being sufficient to obliterate all but a slight passive effect (fig. 1, *B*). On the other hand more than ten times the dose of

adrenalin was required to produce constriction in the normal limb as compared with that for constriction in the denervated limb, e.g., constriction in the denervated limb always occurred with doses of about 0.2 cc. to 0.4 cc., 1: 100,000 adrenalin or less, while from 0.3 cc. to 1.0 cc. 1: 10,000 adrenalin was necessary to produce a similar result in the normal limb.

Cutting the nerves to the limb must produce the result described either by removing the influence of the gangliar dilator mechanism or by modifying the blood vessels themselves so that they do not respond through the medium of the peripheral mechanism. From Gruber's work it appears that after some time has elapsed the dilator response to adrenalin develops in the denervated limb. He assumes that this is due to a recovery of tone. In order to test this theory we conducted the following experiments.

After both the sciatic and femoral nerves of one hind limb were dissected out and secured by loose ligatures, the limb was placed in a plethysmograph tube connected to the double bellows system described above. The nerves were severed and the change in volume of the leg registered every five minutes during the remainder of the animal's life. Every hour the response to a depressor dose of adrenalin was determined. In this way the adrenalin reaction could be studied in direct relation to the condition of relaxation or contraction of the vessel walls.

In the first experiment of this kind the animal (cat, 1.8 kgm.) was anaesthetized with ether and lived for eight hours. The limb dilated at an almost uniform rate for the first five hours after the nerves were cut. Dilatation became slower during the sixth hour and had completely stopped at the end, from which time the volume of the limb remained the same until the eighth hour, when the animal died. The blood pressure remained fairly good until a short time before death. The dose of adrenalin used for testing was 0.2 cc., 1: 100,000. Throughout the experiment this produced a fall in blood pressure, preceded by a slight rise. The limb responded by a short dilatation (which may easily have been due to the preliminary blood pressure rise) followed by a more prolonged constriction until the end of the sixth hour when active dilatation appeared. In other words while the limb was in the process of dilating as a result of denervation adrenalin caused constriction, but when the dilatation from this cause was complete a small amount of active dilatation occurred from adrenalin.

In a second experiment where urethane was given, the cat (2.2 kgm.) lived thirty-three hours. The maximum dilatation was reached be-

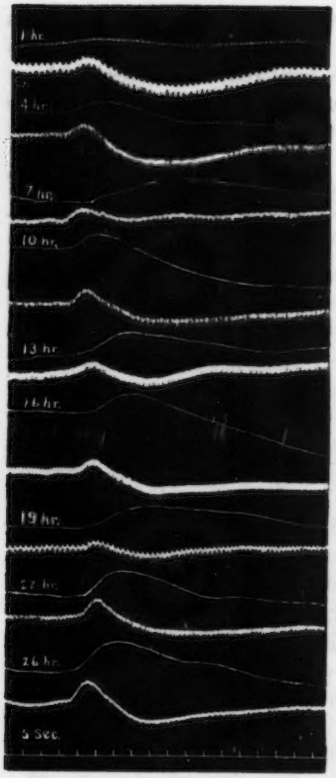

Fig. 2. The response of a denervated limb to a depressor dose of adrenalin during "atonic" and "tonic" conditions. The hours represent the length of time after cutting the nerves. The period of maximum dilatation was reached between the sixth and seventh hours. Up to that time the vessels may be considered "atonic;" after the seventh hour they may be considered "tonic;" 0.5 cc., adrenalin 1: 100,000 was injected in each case. The upper record at each hour represents limb volume, the lower is blood pressure. Cat, 2.2 kgm. Urethane. (Reduced one-half)

tween the sixth and seventh hours. It did not remain long at this level, but constriction soon began, continuing gradually until the twenty-second hour, when it ceased. It remained at this level for the next eight hours. The amount of this remaining dilatation was about one-fifth of the maximum. The dose of adrenalin in each instance was 0.5 cc., 1: 100,000. This usually produced a fall in blood pressure, which was preceded by a rise. During the first five hours adrenalin produced dilatation and constriction of the limb, the dilatation appearing to be largely passive. At the sixth and seventh hours the dilatation became more active and from that time onward the dilator reaction to adrenalin was more pronounced. This was undoubtedly due in part at least to active stimulation, although there was considerable variability in the curves, sometimes the constriction being more pronounced and the dilatation more passive (fig. 2). On the whole it may be said from the two experiments that active dilatation of a denervated limb in response to adrenalin becomes more prominent after the relaxation resulting from denervation has ceased.

In the above experiment we found that a large part of the dilatation resulting from denervation had been recovered from in eighteen hours and that there was little change for the next twelve hours. At this time if the nature of the reaction depends on the condition of tone in the vessels, adrenalin should give good dilatations. In addition to the experiment just described we tried two others. One

hind limb was denervated in each of two cats. Eighteen hours later the animal was again anaesthetized with ether and a study made of the adrenalin response, with the following results:

Cat, 2.2 kgm., 0.3 cc., 1: 100,000 adrenalin caused a similar amount of dilatation in both the normal and denervated limbs. Doses of 0.5 cc. to 1.0 cc., 1: 100,000 adrenalin produced either constriction alone or else dilatation and constriction in the denervated limb. Larger doses produced marked constriction in the same limb. Doses as large as 5.0 cc., 1: 100,000 still produced dilatation in the normal limb, moreover these dilatations were much more pronounced than any resulting in the denervated limb. It took 0.8 cc., 1: 10,000 adrenalin to cause a reversal in the normal limb and then it was not complete, dilatation preceding the constriction.

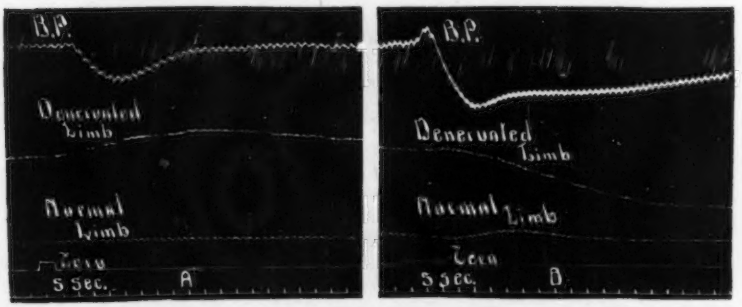

Fig. 3. *A*. Marked active dilatation of a denervated (18 hr.) limb with a small dose of adrenalin 0.2 cc., 1: 100,000. No effect in the normal limb. *B*. Constriction of the same denervated limb with 1.5 cc., 1: 100,000 adrenalin; dilatation of the normal limb. Cat, 2.6 kgm. (Reduced one-half)

Cat, 2.6 kgm., 0.2 cc., 1: 100,000 adrenalin caused a marked dilatation in the denervated limb, but no effect in the normal limb (fig. 3, *A*). Dilatation in the denervated limb, occurred with doses as large as 1.0 cc., 1: 100,000 but 1.5 cc. of the same concentration caused constriction (fig. 3, *B*). Dilatations were not produced in the normal limb until 0.3 cc., 1: 100,000 adrenalin was injected. Dilatation in this limb resulted from doses as large as 0.5 cc., 1: 10,000 adrenalin; however, 0.7 cc. of the latter concentration caused a reversal.

In both experiments the range of dosage for dilatation in the denervated limb was small while quite large amounts of adrenalin were required to bring about reversal in the normal limb. It seems from these experiments that tone may play a part in the response of a denervated limb to small doses of adrenalin. Moreover it appears that the

TABLE 1

A comparison of normal and denervated limbs

ANIMAL	WEIGHT	DURATION OF DENERVATION	DOSE	RESPONSE OF NORMAL LIMB	RESPONSE OF DENERVATED LIMB
	kgm.	*days*	*cc.*		
1. Cat	2.4	7	0.6 A	Dilatation*	Dilatation
			1.0 A	*Dilatation and constriction*	Dilatation
			0.2 B	*Marked constriction*	Dilatation
			0.5 B	*Very marked constriction*	Dilatation
			1.0 B	*Very marked constriction*	*Dilatation and constriction*
2. Cat	3.0	14	0.3 A	Dilatation	Dilatation
			0.4 A	*Constriction*	Dilatation
			0.7 A	*Constriction*	*Constriction*
3. Cat	2.2	15	0.4 A	Slight dilatation	Dilatation
			0.5 B	Slight dilatation	*Marked constriction*
			1.0 B	*Slight constriction*	*Marked constriction*
4. Dog	14.0	22	1.0 A	Dilatation	Dilatation
			1.6 A	Marked dilatation	Marked dilatation
			2.5 B	*Dilatation and constriction*	*Dilatation and constriction*
5. Dog	6.2	31	*0.2 A*	*Nothing*	Dilatation
			0.5 A	*Dilatation and constriction*	Dilatation
			0.2 B	*Dilatation and constriction*	Marked dilatation
			0.5 B	*Very marked constriction*	*Marked dilatation and marked constriction*
6. Dog	5.6	39	0.2 A	Slight dilatation	Slight dilatation
			1.5 A	Dilatation	Dilatation
			5.0 A	*Dilatation and constriction*	Very marked dilatation
			1.0 B	*Dilatation and constriction*	*Dilatation and constriction*

* Unless otherwise stated dilatation means active dilatation.

A = 1: 100,000 adrenalin.

B = 1: 10,000 adrenalin.

peripheral mechanism has a much more limited action than the "gangliar-peripheral" mechanisms when taken together.

After denervation of greater duration. Animals (six dogs and three cats) were studied which had had the sciatic and femoral nerves severed

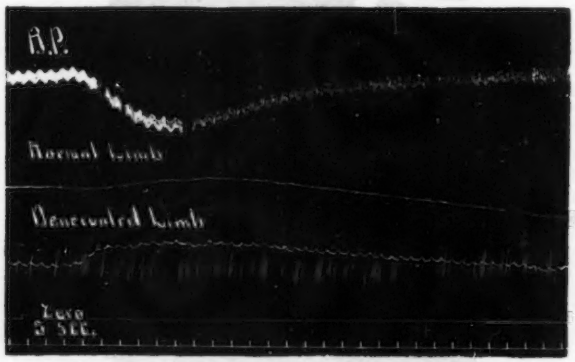

Fig. 4. Dilatation of the hind limb of a cat (2.4 kgm.) to 0.2 cc., adrenalin, 1: 100,000, seven days after denervation. (Reduced one-half)

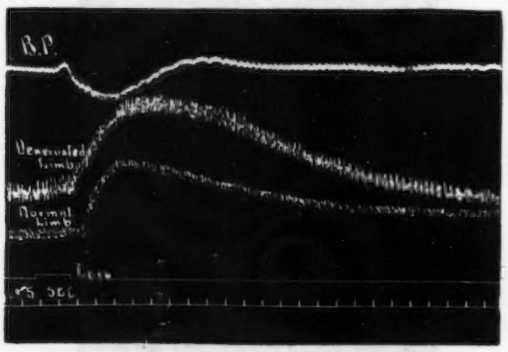

Fig. 5. Dilatation of the hind limb of a dog (14 kgm.) to 0.8 cc. adrenalin, 1: 50,000, twenty-two days after denervation. (Reduced one-half)

in one limb from seven to thirty days before. It can be seen from the following table (table 1) that although the lapse of a week in most cases renders the peripheral dilator mechanism more effective (see figs. 4 and 5), a greater amount of time does not materially increase the

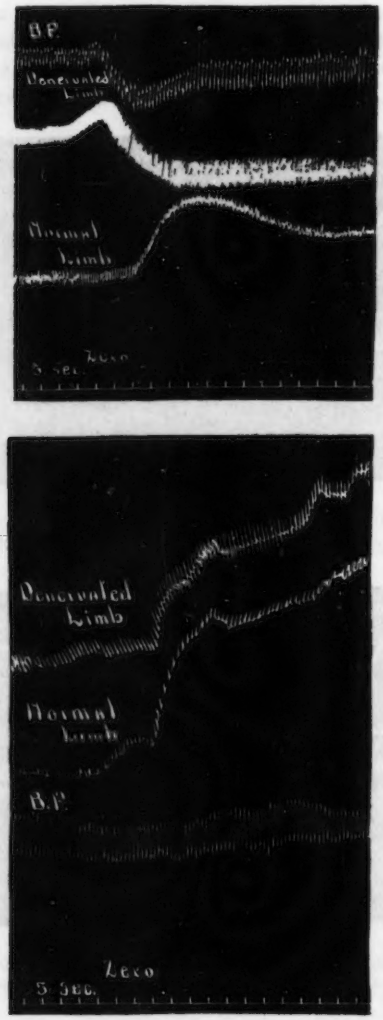

Fig. 6. Reversal of the adrenalin response in a freshly denervated limb by perfusion. Upper record—circulation to limbs intact, 1.0 cc. adrenalin, 1: 10,000 injected into the jugular vein. Lower record—limbs perfused, 2.0 cc. adrenalin, 1: 10,000 injected into the perfusion fluid. Dog 24 kgm. (Reduced one-half)

TABLE 2

Comparison of normal and denervated limbs before and after perfusion

ANIMAL	WEIGHT	DOSE OF ADRENALIN	NORMAL LIMB	DENERVATED LIMB
	kgm.	*cc.*		
7. Dog	17.0	0.5 A	Dilatation	
		0.3 B	Dilatation	
		0.7 B	Constriction	
		1.0 A	*Dilatation*	
		1.0 B	*Dilatation and constriction*	
8. Dog	15.0	1.3 A	Dilatation and constriction	Small dilatation and marked constriction
		0.5 B	Marked dilatation and small constriction	Marked constriction
		0.7 A		*Marked dilatation*
		0.4 B		*Dilatation and constriction*
		3.0 B		*Pure constriction*
9. Dog		0.5 A	Dilatation	Constriction
		0.4 B	Marked dilatation	Marked constriction
		0.7 B	Dilatation and constriction	Marked constriction
		1.0 A		*Dilatation*
		4.0 B		*Marked dilatation*
		1.0 C		*Dilatation and constriction*
10. Dog	7.5	0.4 A	Dilatation and constriction	Dilatation and constriction
		4.0 A	Dilatation and constriction	Marked constriction
		0.5 A	*Dilatation*	*Dilatation*
		1.0 B	*Constriction*	*Constriction*
11. Dog	24.0	0.5 B	Dilatation	Constriction
		2.5 B	Marked dilatation	Marked constriction
		4.5 B	Dilatation and constriction	Marked constriction
		1.0 B	*Marked dilatation*	*Dilatation*
		5.0 B	*Dilatation and constriction*	*Dilatation and constriction*

A = 1: 100,000 adrenalin.
B = 1: 10,000 adrenalin.
C = 1: 1,000 adrenalin.

Limb perfused where italics are used, injections in that case into the perfusion fluid, otherwise into the jugular vein.

effect. In most cases the constrictor mechanism had become less sensitive as compared with that in the normal limb (see animals 1, 2 and 5, table 1). On the other hand, occasionally the dilator mechanism was easily fatigued so that after a few doses the dilator response disappeared or was considerably decreased.

TABLE 3

*Comparison of perfused limbs of animals in table 1**

ANIMAL	WEIGHT	DOSE	NORMAL LIMB	DENERVATED LIMB
	kgm.	*cc.*		
4 Dog denervated 22 days	14.0	2.0 A	Dilatation	Dilatation
		0.4 B	Dilatation	Dilatation
		1.5 B	Dilatation and constriction	Dilatation and constriction
5. Dog denervated 31 days	6.2	0.05 A	No effect	No effect
		0.1 A	Small constriction	Small constriction
		0.5 A	Dilatation and constriction	Dilatation and constriction
		0.2 B	Dilatation and marked constriction	Dilatation and small constriction
		0.2 B		Marked dilatation
		0.5 B		Very marked dilatation
		1.0 B		Very marked dilatation
		0.5 C		Marked dilatation
		0.8 C		Dilatation and constriction

* Injections into the perfusion fluid.
A = 1: 100,000 adrenalin.
B = 1: 10,000 adrenalin.
C = 1: 1,000 adrenalin.

The dilatation of the denervated limb was no better developed in these animals than in some of the responses from a limb denervated but a few hours before (see fig. 2; 7 hr., 19 hr.). However the dilatation was more constant in occurrence and resulted from a greater range of doses. From the very fact that dilatation quite often takes place in the denervated limb from doses larger than those necessary to produce reversal in the normal limb, it seems that a change has taken place in

the myoneural junctions. The constrictor junctions must have lost in sensitiveness or the dilator junctions have gained.

Response of perfused limbs. We have obtained dilatation of both normal and denervated limbs from the injection of adrenalin into the fluid which was perfusing them. A comparison of the perfused normal and denervated limbs injected in this way should help to explain the peripheral dilator mechanism.

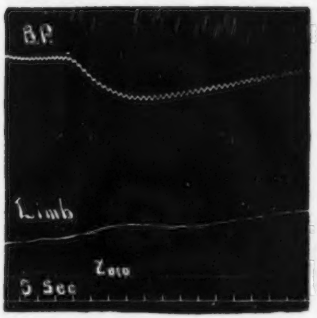

Fig. 7

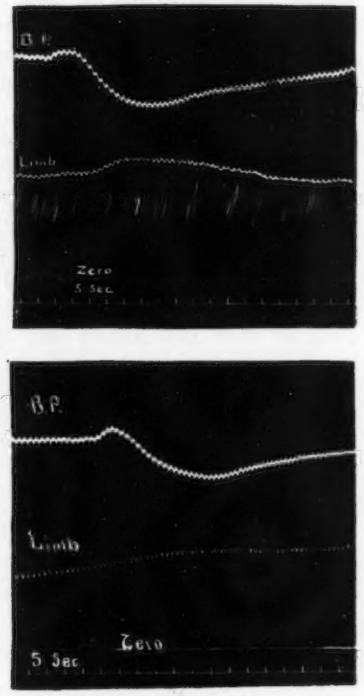

Fig. 8

Fig. 7. Dilatation of a perfused hind limb of a cat by the action of a depressor dose of adrenalin (0.6 cc., 1: 100,000) upon the gangliar portion of the dilator mechanism. Cat 2.4 kgm. (Reduced one-half)

Fig. 8. Dilatation of a hind limb produced by a depressor dose of adrenalin acting upon the gangliar portion of the dilator mechanism. Upper record—response of the hind limb to 0.4 cc. adrenalin, 1: 100,000 injected into the jugular vein, circulation intact. Lower record—response of the same limb to 0.6 cc. adrenalin, 1: 100,000 injected into the jugular vein. (Reduced one-half)

By perfusion of a recently denervated limb an immediate change in the response to adrenalin is brought about, so that dilatation instead of constriction is easily produced (fig. 6 and table 2). This change is similar to that occurring in a denervated limb with normal circulation several hours after denervation (figs. 2, 3, 4, 5 and table 1). The peripheral response to adrenalin in perfused normal and denervated limbs is essentially the same when carried out simultaneously in one animal (animals 10 and 11, table 2). On the other hand there is greater variability in the response of a perfused limb which has been denervated for several days. In one case the constrictors were easily fatigued so that after a few doses of adrenalin they could not again be brought into action except by a dose of 0.8 cc., 1:1,000 (animal 5, table 3). In another case even perfusion did not bring about dilatation in an animal which had shown no active dilatation with intact circulation (dog, limb denervated eight days). The normal limb gave dilatation before and after perfusion but this is an exceptional case in our experience.

"Gangliar" dilatation from depressor doses. Gruber (9, p. 311) failed to obtain dilatation of a perfused limb from the injection of depressor doses of adrenalin to the general circulation. He infers that the gangliar effect is produced only by pressor doses. We have been able to show in two experiments that depressor doses of adrenalin can bring the gangliar mechanism into action. In both animals a slight increase in the dose was necessary, but the blood pressure response was a pure fall or else a slight rise and decided fall. The animals were cats weighing 2.4 kgm. and 3.0 kgm. In the first, 0.6 cc., 1:100,000 was required after perfusion (fig. 7). In the second, 0.4 cc., 1:100,000 caused dilatation before, while 0.6 cc., 1:100,000 was required after perfusion (fig. 8). When perfusion had gone on for some time even larger doses of adrenalin were required to produce dilatation.

DISCUSSION

The relation of tone to the reversal of adrenalin effects. Recognizing the fact that adrenalin may cause dilatation through both gangliar and peripheral action, we are confronted with the question as to the normal site of dilator action. It has been shown that cutting gangliar connection with the limb in a majority of cases prevents the dilatation of that part. Gruber (9, p. 307) maintains that this is due to a loss of tone in the vessels. In order to understand the development of the tone theory, we should first consider the work of Cannon and Lyman

(10) who were the first to suggest this interpretation for the opposite effects of depressor doses of adrenalin. Their view was reached by the exclusion of other possibilities, viz., (1) central source, (2) blocking of vasoconstrictor impulses, (3) stimulation of vasoconstrictor and vasodilator nerve endings. Their exclusion of the third possibility was on account of the meagre evidence for the existence of vasodilator nerves in the sympathetic system. They found that the blood pressure response was changed to a rise if the tone had been lowered sufficiently by overheating, separation from the central nervous system or by extreme action of the depressor nerve. They attributed vasodilation and vasoconstriction to opposite actions of adrenalin according to the state of the muscle—relaxation when tonically shortened, contraction when relaxed.

Gruber's conclusions were reached because of his inability to obtain dilatation in a freshly denervated limb and the recovery of the dilator response in a limb a few days after denervation. He attributed the reappearance of the dilator reaction to a restoration of tone. It might also be due to a loss in sensitiveness of the constrictor myoneural junctions.

Let us consider, first, the question of tone. In all of our experiments with recently denervated animals the reactions to adrenalin were studied within thirty minutes after denervation and were continued for one or two hours. We have shown above that the maximum dilatation is not reached until the sixth hour after denervation so that those studies were made during the period of steady relaxation. Within this time the usual adrenalin response is constriction, afterwards the reaction begins to reverse (fig. 2). It is not that the vessels have suddenly dilated to their limit and cannot expand further, because they only gradually reach this stage after six or seven hours. Moreover they do not appear to dilate to the limit at any time as a result of denervation because while they are in this state of maximum relaxation, depressor doses of adrenalin often cause further dilatation (fig. 2). We may draw the conclusion that while relaxation is going on the vasodilator myoneural junction is not so easily brought into action and that the constrictor effect therefore predominates.

The state of relaxation seems to affect only the adrenalin receptive substance. Active dilatation of a denervated limb in which the vessels are relaxing can easily be produced by a substance from ox pituitaries (fig 9). In the same animal depressor doses of adrenalin usually caused constriction of the denervated limb (fig. 1).

After denervation of greater duration. A few days after cutting the nerves to a limb, the latter has regained its power to dilate in response to adrenalin so that it does as well as the normal limb. This might be explained by the recovery in tone, but a large part of the tone has been recovered within twenty-four hours, so that the reaction at the twenty-fourth hour should not differ much from that several days later. But it does differ in this respect that in denervations of longer duration it requires much larger doses of adrenalin to cause constriction; in other words, there is a larger range of dosage producing dilatation. In fact a larger dose than that required for the normal limb is needed to bring about reversal in the majority of cases (table 1). Gruber (9, p. 310) also found this to be true. One can interpret this either as a loss in sen-

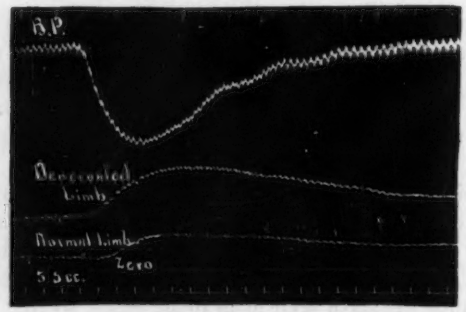

Fig. 9. Dilatation of a freshly denervated limb, produced by a depressor substance obtained from pituitary glands. Cat. (Reduced one-half)

sitiveness of the constrictor junctions or a gain in sensitiveness of the dilator junctions. The tone theory, however, does not appear to account for this point.

In regard to the question of variation in sensitiveness of the myoneural junctions we have the work of Elliott (12), which indicated that all muscles thrown into contraction by adrenalin have their irritability to this substance increased by denervation. However we have no proof that dilator junctions would be thus affected.

Effects of adrenalin in perfused limbs. A number of investigators have studied the response of various perfused organs to adrenalin with variable results. This would be one of the best methods of proving the existence of vasodilator nerves in the sympathetic if active dilatation could be so obtained.

Employing the change in rate of venous outflow to indicate the vasomotor response Salvioli (14) and Brodie and Dixon (15) obtained only constriction in the hind limb when adrenal extract or adrenalin was added to the perfusion fluid. The latter experimenters found this to be true even in limbs which had been denervated two or three months before. Pari (16) repeatedly obtained an increased outflow from the limb in one experiment when a perfusion of 1: 500,000 adrenalin was used, but he inferred that this was due to decomposition products.

Langendorff (17) from his results with rings of coronary arteries concluded that they possessed sympathetic vasodilators which were stimulated by adrenalin. His results were confirmed by Cow (18) and Park (19). Brodie and Cullis (20) from experiments upon perfused hearts concluded that the main cause of adrenalin dilatation was the excitation of vasodilator "nerve-endings."

Langlois and Desbouis (21) obtained constriction in the lung vessels with large doses, 1.0 mgm., and dilatation with small doses, 0.05 mgm. Similar results on perfused lungs were described by Tribe (22).

Other organs have given dilatation from dilute adrenalin perfusing them. For instance, the kidney and the intestine have been found by Ogawa (23) to react in this way. But so far as we know the limb has not been found to react thus when perfused except in the one experiment of Pari (16) and in experiments by Ogawa (23) on the rabbit in which he sometimes obtained dilatation following constriction, but never primary dilatation.

We found it easy to produce dilatation by the injection of adrenalin into the fluid perfusing a limb. Whether the nerves had been cut or not seemed to make no difference in the reaction (table 2).

Why should perfusion reverse the reaction of a denervated limb? Does it mean that perfusion of vessels which were previously relaxing causes them to begin to contract and thus produces the reversal? That might be the condition in perfusion with low pressure (20 mm.) but in a number of our experiments we have doubled or tripled the pressure without materially reducing the dilator response to adrenalin. Moreover Tribe (22) found in the perfused lung that with high pressure it was easier to obtain dilatation than constriction.

Another observation which suggests an explanation of the results just described is the increase in the range of doses of adrenalin which will cause dilatation in both normal and denervated limbs. The interpretation which this seems to suggest is that perfusion renders the constrictor myoneural junction less sensitive or the dilator junctions more

sensitive. We have found that it takes much larger doses of adrenalin to bring about constriction in a perfused limb than it did while the circulation was intact, whether it be a denervated limb or one with nervous connections (table 2). For example: whereas 1.0 cc., 1: 10,000 adrenalin injected into the jugular vein before perfusion caused constriction in the denervated and dilatation in the normal limb, 2.0 cc., 1: 10,000 (a dose more than four times as great, considering the limited circulation of the perfusion fluid) injected into the perfusion fluid caused marked dilatation in both limbs (fig. 5). Mechanical effects from the injection were compensated for by a simultaneous withdrawal of an equal quantity of perfusion fluid.

The work of Meyer (13) supports the idea that Ringer's solution modifies the sensitiveness of blood vessels to adrenalin. He found that artery rings kept for some time lost their sensitiveness to adrenalin from day to day and after it had disappeared an opening shock still produced contraction. His results might be due to the changed medium in which the preparations were kept rather than to denervation.

Dilatation from the stimulation of "gangliar" and "peripheral" mechanisms. Before we enter into the discussion of the relative importance of the "gangliar" and "peripheral" mechanisms we wish to call attention to the results of Gruber (9, p. 311), in which he failed to obtain dilatation of a perfused limb from the injection of a small dose of adrenalin into the general circulation. Because the same dose caused dilatation in the intact limb he infers that the dilatation from small doses must be due to peripheral instead of gangliar action. He says:

> If adrenalin exerted its influence entirely through a vasodilator center, it should produce the same results in these two cases where the only difference in the conditions of the limbs is that one has and one has not the circulation intact.

This is a very serious difference and might easily account for the increase in the dilator threshold. Oxygenated Ringer's solution or even oxygenated defibrinated blood cannot be expected to fulfil the function of normal blood in all respects and indeed this was not the only difference, for the occlusion of the abdominal aorta interferes with the circulation to the ganglia of the nerves supplying the limbs. The latter condition alone might necessitate a larger dose of adrenalin. If both of these conditions were operative, the dose required would probably in many cases be a pressor dose. However, we have been able to show in two experiments that depressor doses can bring the gangliar mechanism into action. These render unnecessary the assumption of periph-

eral action to account for dilatation resulting from small doses of adrenalin.

We are not in a position to say which is more important in producing dilatation normally, the "gangliar" mechanism or the myoneural junction. It has been possible in some animals to obtain the same amount of dilatation by the action of adrenalin upon the gangliar portion of the mechanism alone (limb perfused, adrenalin injected into the jugular vein) as occurred from the injection of the same quantity when the circulation of the limb was intact. In many cases, however, larger doses are required to produce equal response in the limb when only the gangliar mechanisms are affected as compared with the condition where both gangliar and peripheral portions might be brought into action. This may easily be attributed to the reduced circulation to the ganglia brought about by clamping the aorta high in the abdomen, but the fact that the peripheral dilator mechanism can be brought into action rather easily under many circumstances indicates that it may well be as important as the gangliar dilator mechanism. At any rate we seem justified in concluding that sympathetic vasodilators to the limb exist and that they are sensitive to adrenalin at the "gangliar" and "peripheral" ends.

We wish to thank R. S. Lang for assistance in this research.

SUMMARY

1. While a limb is dilating from denervation adrenalin produces an increase in volume with difficulty, but while the reverse change is taking place the dilator effect of adrenalin begins to reappear.

2. After denervation of a limb, of greater duration, the dilatation from adrenalin occurs from a greater range of doses than is the case in the normal limb.

3. The peripheral action (dilatation) becomes similar in both normal and denervated limbs after perfusion. Under these conditions also dilatation occurs with a greater range of doses.

4. Depressor doses of adrenalin can cause dilatation of a limb by action on the gangliar mechanism.

5. Adrenalin acts on both "gangliar" and "peripheral" mechanisms in producing dilatation of the hind limb.

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CONSTRICTION FROM ADRENALIN ACTING UPON SYMPATHETIC AND DORSAL ROOT GANGLIA

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In the preceding research it has been shown that adrenalin can produce dilatation in a limb by acting upon a "peripheral" mechanism as well as upon a gangliar mechanism. We have been able to show that the constrictor action of this hormone is not confined to the myoneural junction. Although the gangliar response is not easily obtained, it has been found often enough to draw our attention. The methods employed were those described in preceding researches.

All experiments showing constriction from gangliar action must necessarily be those in which the organ tested is completely cut off from the general circulation in order to prevent the peripheral action of adrenalin.[1] Perfusion experiments in which anastomoses to the organ are cut off, satisfy this condition.

Constriction of the limb. Six animals out of nineteen furnished evidence of gangliar constriction in the hind limb. One dog (16 kgm.) and one cat (3 kgm.) gave constriction followed by dilatation when adrenalin was injected into the jugular vein; the first with a dose of 4 cc., 1: 20,000 adrenalin, the second with a dose of 5 cc., 1: 5,000 adrenalin. In each animal both sympathetic and dorsal root ganglia were intact. On the other hand similar experiments with six dogs and three cats gave no constriction although the usual dilatation could be obtained.

From sympathetic ganglia. Two cats gave positive evidence of a constrictor action of these ganglia by the direct application of adren-

[1] Salvioli (Arch. ital. de biol., 1902, xxxvii, 384) perfused the limb of a dog, with the nerves intact. Adrenal extract was injected into the jugular vein and the volume change in the limb was studied by the venous outflow. He usually obtained no change in the flow but occasionally there was a small decrease in the outflow. This was believed to be due to the escape of adrenal extract into the limb because the decrease was not synchronous with the rise in blood pressure; in fact the pressure had returned to normal before the limb changed.

alin to them. In the first, a 1: 100,000 adrenalin solution produced only dilatation while a 1: 10,000 solution caused steady marked constriction. In the other a 1: 1,000 solution caused a dilatation followed by constriction. Three animals gave no constriction. The first (a cat) was tried by dropping adrenalin upon the sympathetic ganglia. On the last two (dogs) the dorsal root ganglia had been removed, adrenalin being given by the jugular vein.

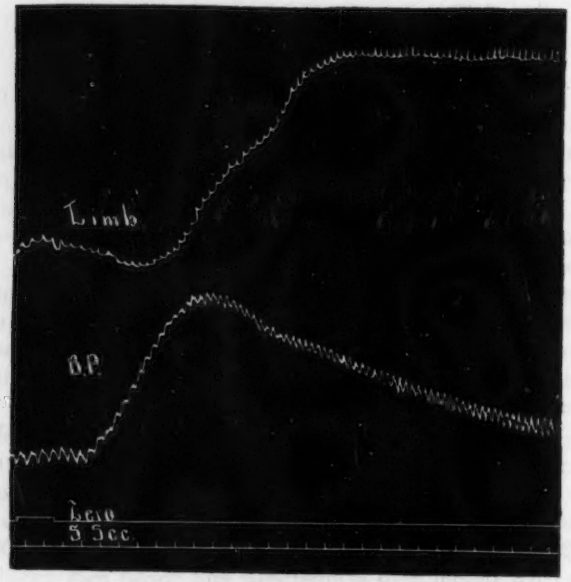

Fig. 1. Constriction and dilatation of a perfused limb from the injection of 4 cc. adrenalin, 1: 5,000 into the jugular vein. All sympathetic ganglia supplying the limb had been destroyed. Dog 21.6 kgm. Reduced ⅓.

From dorsal root ganglia. Of the animals (seven dogs) in which the sympathetic ganglia to the perfused hind limb had been destroyed, only one responded by constriction when adrenalin was injected into the general circulation (fig. 1). Direct application of adrenalin to the dorsal root ganglia in one of two cats caused constriction in the hind limb (fig. 2). In almost all of the animals studied whether giving gangliar constriction or not, dilatation from adrenalin was obtained.

We may say, in general for the hind limb, that the effect of adrenalin on the ganglia is preëminently dilator and that the constriction from this source is insignificant.

Constriction of the intestine. Constriction of a gangliar source was more common in the intestine than in the limb. A response of this

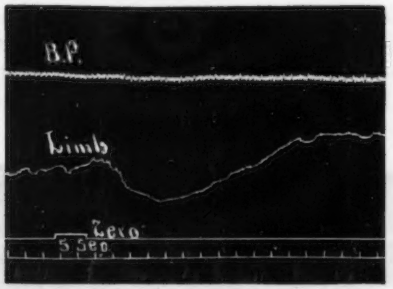

Fig. 2. Constriction of the hind limb resulting from the direct application of 1: 1,000 adrenalin to the lower lumbar dorsal root ganglia. Dog 16 kgm. Reduced ½.

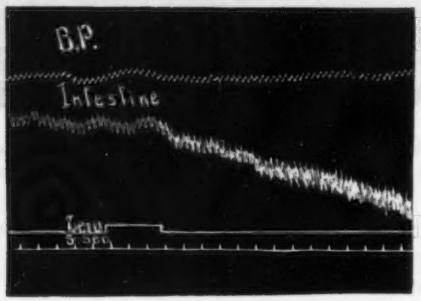

Fig. 3. Constriction of the intestine produced by direct application of 1: 1,000 adrenalin to the twelfth and thirteenth dorsal root ganglia. Dog 11 kgm. Reduced ½.

sort was obtained in six out of thirteen animals. Moreover the number of constrictions obtained in the same animal was much greater in the case of the intestine than in the experiments with the limb. In the latter there would often be only one or two constrictions throughout the whole experiment.

Three dogs whose splanchnic nerves had been cut gave positive evidence of gangliar constriction. The intestinal loop was perfused and the adrenalin was injected into the jugular vein. Both constriction and dilatation occurred whenever the intestine responded by constriction.

Intestinal constriction was also produced by the direct application of adrenalin to the dorsal root and superior mesenteric ganglia.

In a cat although dilatation only had been produced by the application of 1: 1000 adrenalin to the twelfth and thirteenth thoracic dorsal root ganglia in three instances, in a fourth the same concentration produced constriction followed by dilatation.

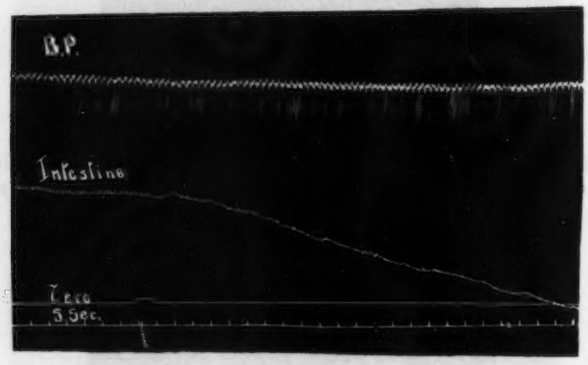

Fig. 4. Constriction of the intestine from direct application of 1: 1,000 adrenalin to the superior mesenteric ganglion. Dog. Reduced ½.

In a dog, a 1: 1,000 solution produced dilatation alone, constriction alone (fig. 3) or constriction followed by dilatation.

Marked constriction of the intestine was caused in another experiment by treating the superior mesenteric ganglion with 1: 1,000 adrenalin chloride to which a little pure adrenalin had been added (fig. 4).

Additional evidence that the superior mesenteric ganglion is a source of constriction was obtained in one animal by the use of nicotine. Before nicotine, adrenalin caused constriction followed by dilatation of the intestine. Intravenous injection of nicotine ruled out both the constriction and dilatation.

Thus the gangliar effect of adrenalin as far as the intestine is concerned is largely dilator, although it is sometimes a source of constriction.

SUMMARY

1. Adrenalin occasionally produces constriction in the hind limb by its action upon the sympathetic and dorsal root ganglia.

2. Constriction of the intestine is sometimes produced by adrenalin acting upon the superior mesenteric and dorsal root ganglia.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH, NO. 92, AND FROM THE ANATOMICAL LABORATORY OF THE NORTHWESTERN UNIVERSITY MEDICAL SCHOOL, NO. 62.

THE MULTIPLE SENSORY ACTIVITIES OF THE SO-CALLED RHINOPHORE OF NUDIBRANCHS

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PRELIMINARY

A striking feature of nudibranch molluscan morphology is the pair of short, robust dorsal tentacles which are commonly perfoliate or ringed and which in many species are retractile. These distinctive tentacles are known as "rhinophores" and it is tacitly assumed that they are, as the name indicates, specialized organs of the olfactory sense.

The only evidence that would at all favor the assigning to these structures of an exclusive or predominating olfactory function has been presented by Graber ('89). This experimenter brought oil of rose near the head of Chromodoris elegans and observed the withdrawal of the rhinophores to be quicker and more vigorous than that of the oral tentacles. It is significant, however, that Graber states emphatically that the post-branchial region is the most sensitive part of the body. By whom the word "rhinophore" was coined and in what sense it was first applied is not clear; malacologists to whom I have referred this query are unable to trace its origin.

It thus appears that the propriety of the convenient term rhinophore lacks appropriate experimental support. For this reason nudibranchs were subjected to experimentation designed to test their sensory potentialities ('17).[1] Unless otherwise stated the present account deals with the Bermudian Chromodoris zebra Heilprin.

[1] This work was made possible through the hospitality of Professor E. L. Mark, Director of the Bermuda Biological Station, and the liberality of the trustees of the Humboldt Fund of Harvard University.

OBSERVATIONS[2]

Tactile stimulation. When a rhinophore is touched lightly with a glass rod it is jerked back precipitately within its protecting collar. The sensitivity of the rhinophore to gentle stimulation is astonishing and the explosive type of response is, within wide limits, independent of the strength of the stimulus. Fatigue comes on but slowly, responses of somewhat diminished intensity being readily obtained after fifty successive stimulations at ten-second intervals.

The oral tentacles, gill plumes and the general body-surface all respond to tactile stimulation. It is rather unsatisfactory to list the several regions of the body in the order of their sensitivity, for the types of responses are not all comparable. It appears, however, that the so-called rhinophore is the most sensitive part of the body to this kind of stimulation and considerably more so than the oral tentacles.

Thermal stimulation. The head region and especially the oral tentacles react distinctly to water at 40° to 50°C. applied with a pipet. The rhinophores, on the contrary, give faint and rather doubtful responses except to temperatures as high as 50°C.

Rheotrophic stimulation. I am informed by Dr. W. J. Crozier[3] that the rhinophore of Chromodoris is of prime importance in effecting orientation to water currents. Animals from which these structures have been removed do not orient at all, or do so with extreme slowness and hesitancy; when only one functional rhinophore is left, circus movements are simulated.

Chemical stimulation. Equal volumes of various chemical solutions were applied from a constant distance with a pipet; with care tactile stimulation by the stream can be avoided. Solutions of 1 M maltose, or sucrose, or M/2 lactose were without effect upon all parts of the body, although 3 M glycerin did evoke general responses. Several alkaloids had very weak effects or none at all. Alcohols and organic acids in concentrations of M/10 called forth strong general responses. The chlorides of the alkali metals Na, K, NH_4 and Li likewise stimulated the body in general, the rhinophores and oral tentacles, however, showing the greatest sensitivity. Solutions of substances which produce in man the taste sensations recognized as acid (HCl), bitter (picric acid), salty (KCl), and alkaline (KOH), were applied in various con-

[2] An extended account of the behavior and sensory physiology of Chromodoris is in preparation by the writer in collaboration with Dr. W. J. Crozier.

[3] Unpublished observations.

centrations. All responses gradually weaken with increasing dilution, but the gills fail usually before other parts. There was some evidence that the oral tentacles were more sensitive to picric acid than were the rhinophores.

From the foregoing tests it becomes evident that the rhinophore is not only extremely sensitive to chemical stimulation of diverse sorts, but that this sensitivity is only second to, if indeed it does not equal, that of the oral tentacles, which from their position might be suspected *a priori* of a specialized gustatory or common chemical function.

Olfactory stimulation. Saturated solutions of various essential oils[4] were prepared by shaking with sea water. These solutions were applied gently by a pipet to the several regions of the body surface.

The rhinophores of Chromodoris react vigorously to such stimulation but, so far as one can judge from the dissimilarity of the responses, other parts of the body appear to be equally sensitive. When a drop of oil is held for some time midway between the rhinophores no response ensues. If the rhinophore or general body surface be touched gently with a drop of pure oil, the response is weaker than to a saturated aqueous solution; in this case the number of sense organs stimulated undoubtedly is a complicating factor, yet it suggests further that the response is one to an olfactory stimulus rather than to an irritative or "smarting" one.

Chromodoris was also tried with solutions in which marine invertebrates had partially decomposed. Such a test is undoubtedly complicated by the presence of certain chemical substances not of an odorous nature, yet it at least simulates the type of olfactory stimulation met by the animal in a state of nature.

Water contaminated by a dead crab or by the viscera of a holothurian (these solutions being decidedly odoriferous to the human sense of smell) stimulated strongly all regions of the external body surface. Water from decaying coral or Onchidium likewise evoked responses from the general body surface except the gills. On the contrary, another specimen of coral water which did not smell particularly strong, was found to stimulate the rhinophores and head region in general but not the rest of the body; the sensitivity of the several head structures was, however, equal.

Facelina goslingi possesses long oral tentacles and rhinophores. Its entire body surface, including the rhinophores, is responsive to tactile

[4] Bergamot, cassia, clove, juniper, origanum, pennyroyal and thyme. Anilin oil and carbon bisulpide were used also.

and chemical stimulation. The animal is exceedingly active, climbing the walls of a container and swimming on the surface film. It was found that merely holding a drop of oil near the body of a crawling animal did not provoke a response, whereas actual contact (eliminating tactile complications) would do so. When stimulated with solutions the non-retractile rhinophores react by a lashing withdrawal and more vigorously than do the oral tentacles; this is the only clear case recorded of a superior reactivity of this organ to odorous substances. Oil of pennyroyal, carbon bisulphide and anilin oil proved to be more efficient than the oils of bergamot, cassia, cloves, juniper and origanum.

Elysia crispa is a small nudibranch which also tries to swim on the surface film. When crawling on the substrate its anterior half often loosens its attachment and is elevated and waved about, the posterior half still locomoting the while; this attitude is favorable for detecting the effect of stimulation. To light touch alone there is a slight retraction of the extended body. The odorous oils are more stimulating; to bergamot, cassia, cloves, juniper, origanum, pennyroyal and thyme applied to the rhinophores the retraction is noticeably sharper and of greater amplitude. Carbon bisulphide and anilin oil are without effect; this is in decided contrast to the results on Facelina recorded in the last paragraph. The general body surface is likewise responsive to these olfactory agents and to ordinary chemical stimulation as well; to solutions of the essential oils in sea water, the head region was more sensitive than the remainder of the body.

Fiona marina, a nudibranch found in the floating gulf weed, Sargassum, is sensitive on its rhinophores and body to essential oils and to other general chemical stimulation.

DISCUSSION

There is nothing in the foregoing tests which specifically connects the rhinophores with olfaction. Rather they appear to respond to various sorts of sensory stimuli, and to share this sensitivity liberally with other regions of the body. In Facelina alone is there a sharper rhinophore reaction, and even in this case it is not certain that the vigor of response to supraminimal stimuli is the expression of greater inherent sensitivity.

We know nothing concerning the existence of diverse sensory endings in nudibranchs for the reception of chemical stimuli. Presumably they

do not exist (cf. Smallwood, '12).[5] Under such conditions it is futile to speak of a sense of touch, taste, smell and so on. It is true that many invertebrates are capable of responding to a variety of sensory stimuli and that, furthermore, such responses are often more or less accurately adjusted, in a quantitative manner, to the quality, strength or frequency of the stimulating agent; yet the occurrence of differentially selective responses to definite stimuli is limited. In certain instances, to be sure, specific sense organs (e.g., eye; otocyst) are recognized which respond exclusively or chiefly to particular stimuli; more usually identical responses follow widely diverse sensory excitants. Much of the older results regarding the existence and localization of differential sensitivity is uncritical and untrustworthy.

There is no reason to believe that organs structurally uniform and capable of responding identically to a variety of qualitatively different stimuli, are able to analyze, thereby producing qualitatively distinct sensations. In other words, it can not be assumed that such an animal is capable of differentiating various stimuli intuitively, nor that as a functional adaption individual receptive elements, morphologically indistinguishable, have acquired the capability to analyze. Such assumptions are opposed to the principle of specific energies, which is by far the safest guide in the study of invertebrate sensory potentialities.

Histologically identical sense organs, however, due to topographical arrangement, may in a practical way chiefly serve physiologically distinct functions. Thus, certain receptors about the mouth are said to be useful mainly for proving food, those at the entrance of the respiratory chamber, for testing the quality of the respiratory medium, and so on. This method of logical assignment of function is not without danger; for example, the insect antenna has been persistently associated especially with olfaction, yet McIndoo ('14) finds that this classic example will not stand the test of experimentation.

If the foregoing conclusions be sound they are of interest in view of a recent contribution by Copeland ('18) who, reflecting the tone of a

[5] It is, however, not impossible that what is accepted in certain instances as a specific form of nerve terminal, such as a free nerve ending, might upon close study be amenable to division into morphologically distinct types. On the contrary, the really significant and fundamental qualitative differences (histologic or chemical) could easily be so subtle that our crude methods must ever fail to detect them. Furthermore, although it is perhaps true that even the simplest metazoans which have nerve terminals at all have them somewhat differentiated, the fact remains that in this field as a whole the anatomic evidence lags.

certain group of writers, reports on what is termed the olfactory reactions of marine snails. That the animals studied sought food through the action of a chemical sense located chiefly in the osphradium, although the external body surface was also responsive to a lesser degree, seems certain. But the contention is pressed further: that, since the osphradium responds to weak concentrations of oyster juice and the rest of the body to relatively high concentrations, the osphradium must be of an olfactory nature and the general body surface gustatory.

This conclusion by no means follows. In the absence of differential receptors a separation into smell and taste on the basis of sensitivity alone is no more defensible than to split into two categories the tactile sense of the human finger and back.

In man, to be sure, the diverse sensitivity of smell and taste to substances that stimulate both is one distinguishing characteristic (Parker and Stabler, '13), but there are other more germane criteria of a qualitative nature. Moreover, to argue by analogy from man to lower vertebrates with respect to these sensory activities is not especially hazardous, for the homologies of innervation and structure are not only certain but the one mechanism or the other can be eliminated experimentally with precision (by section of nerves, plugging of nostrils, etc., Sheldon, '11; Parker and Sheldon, '13). In invertebrates, however, where not even the existence of separate, appropriate end organs has been demonstrated, the argument by analogy exceeds the limits of legitimate deduction.

Finally, although such substances as fish juice or oyster juice combine elements of taste and smell for human sensibilities, we must distrust the capability of the invertebrates in question to analyze chemical stimuli as discrete sensations, and it would therefore appear safer to avoid referring in their cases to a sense of taste and smell at all (or even to a common chemical sense, since this term is preëmpted in vertebrate physiology). What such animals possess can perhaps be designated a "general chemical sense."

SUMMARY

Experimental evidence does not substantiate the popular impression that the nudibranch rhinophore is a specialized organ for detecting odorous substances. It is, rather, a generalized organ responsive to a variety of sensory agents.

In the absence of differential receptors and the probable resulting failure to translate stimuli into discrete 'sensations,' it is safer to avoid terms like taste or smell, especially with the implication of any similarity to corresponding human sensations. Since we comprehend sensory processes only in terms of human experience, there is, however, no objection to retaining such convenient phrases as "olfactory stimulus."

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ACAPNIA AND SHOCK[1]

VIII. The Veno-Pressor Mechanism

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There must be some mechanism controlling the supply of blood from the veins to the heart and regulating the variations needed during rest and exercise. This mechanism must adjust the venous pressure to dilate the right heart during diastole, neither over nor under filling the ventricle.

The variations in the volume of the venous return required and provided are enormous. During bodily rest the venous pressure in man in the erect position rises scarcely above the axilla. During exercise, when the heart is pumping out of the venous system a greatly increased volume of blood, the venous column instead of being reduced is many centimeters higher than in bodily rest (1 and 29).

What is the controlling mechanism producing this rise? Is its action direct or indirect, primary or secondary? What is the influence, nervous, mechanical or chemical, which adjusts its activity to the general needs? Is the locus of stimulation central or peripheral? Undoubtedly the universal answer today would be included under: Regulation by the vasomotor nervous system.

The purpose of this paper is to present facts indicating that, in addition to indirect vasomotor nervous influences (2), *there is a peripheral chemical control of the volume of the venous return*, and reasons, based on

[1] We use the term "shock" merely in the sense of "depression of vitality." Surgical shock is the depression following anesthesia and operation; traumatic that due to trauma, and toxaemic that incident to toxaemia. Even ten minutes of anesthesia and a minor operation may depress vitality to some extent. A major operation usually depresses greatly for hours or days. Our problem is the causes and modes of these depressions, i.e., shock in all degrees, small as well as great. Shock as a moribund or almost moribund state, the sense in which most investigators seem now to use the term, appears to us to be a conception which for purposes of investigation is both artificial and sterile.

previous papers from this laboratory, for holding that it is this chemical control which is the chief factor in the high venous pressure of muscular work and in the lowering of venous pressure induced by acapnia and itself causing the circulatory depression following anesthesia and surgical operation.

The nature of the problem is seen when we compare the circulatory effects of muscular exertion with those of mental strain. The latter causes a very marked rise of arterial pressure—a rise of 30 or 40 mm. of mercury, and once even 80 or 90 mm. in several subjects has been observed in this laboratory during a mere lecture on the blood gases. We suppose this high arterial pressure to be an almost pure vasomotor nervous effect, aided perhaps by some adrenal or other internal secretion. There is however no rise of venous pressure—a fact which becomes highly significant when we consider that the abundant chemical stimulations, altered blood gases and especially increase of CO_2, involved in muscular effort are lacking in mental strain.

On the other hand physical exertion—running, stair climbing or riding a stationary bicycle—sufficient to cause a rise of 30 to 40 mm. of mercury in arterial pressure always induces also a rise of the venous pressure so marked that the hand and arm may be lifted many centimeters above the axilla before the veins collapse. Even when every allowance is made for the accessory parts played during physical work by respiration and by muscular movements in pressing the venous blood onward toward the heart, there seems to be in these facts ground for suspecting the existence of some factor in the circulation acting as a powerful accessory to the vasomotor nervous system in accelerating the venous return, and regulated by chemical rather than by nervous influences (cf. Boothby, 3).

As regards the depression of the circulation induced by anesthesia (4) and surgical operation and by acute disease,—conditions in which the CO_2 content of the blood varies in the opposite sense to that occurring in muscular work,—the evidence brought forward in previous papers from this laboratory points clearly to a lowering of venous pressure and decrease of the venous return as the primary factor (1), (5), (6). And yet in such conditions the vasomotor nervous system is not inactive but rather exerted to increasing and finally maximal effort in constricting arterioles in the endeavor to maintain arterial pressure. It thus strives in normal fashion to compensate the decreasing output of the heart. It acts exactly as after hemorrhage (7).

In harmony with this is the fact that the circulatory effects of even

slightly excessive respiration either natural or artificial in animals under experiment are seen first in the change from well filled to empty jugulars, i.e., decreasing venous return, long before arterial pressure is impaired.

Pointing in the same direction is the fact that voluntarily forced breathing in man has a markedly depressant action on the circulation. The effect is not upon the heart nor upon the vasomotor mechanism, for the heart is accelerated and arterial pressure is not usually lowered. The circulation is rendered slower; and this, as has been shown in a recent paper from this laboratory, is due to a decrease in the venous return to the heart (8). The cause is clearly the reduction in the CO_2 content (or perhaps fundamentally in the C_H) of the blood, for the disturbance does not occur when the forced breathing is performed into a bag so as to prevent excessive pulmonary ventilation.

Conversely an accumulation of CO_2 in the blood causes an abnormally high venous pressure, a dilatation of veins and an exaggeration of the volume of the venous return out of all proportion to the effect on arterial pressure. One of us has had a considerable and unique experience bearing on this matter in connection with tests of the so-called self-contained oxygen mine rescue apparatus (9). In men wearing such apparatus and walking at three or four miles an hour the differences in the effect of insufficient oxygen without accumulation of CO_2 and the effect of large accumulation of CO_2 (3 to 7 per cent) with ample oxygen are very striking. Under the low oxygen the effects are manifested as a grey cyanosis and fainting. Under high CO_2, with ample oxygen, the color of the skin is good, there is intense throbbing headache, the world turns black before the eyes, the gait becomes staggering but the legs do not usually give way. The most striking symptoms however are the indications of greatly elevated venous pressure. The top of the venous column may be at the level of the face or even higher. The superficial veins of the neck and face are enormously distended, both by this pressure and by the relaxation of their own walls. The picture is in this respect like that in the CO_2 acidosis of some patients with renal disease in whom so far as we can judge the heart is not sufficiently affected to explain the venous congestion.

Haldane (10) independently notes (in gassed soldiers) grey cyanosis without rise of venous pressure from oxygen want without excess of CO_2, and "full blue cyanosis and venous engorgement" when excess of CO_2 is added. He does not mention venous engorgement with bright pink lips from high CO_2 and ample oxygen. (Doctor Haldane himself was the first subject in whom I saw this phenom-

enon (at Guy's Hospital, London, in 1913). The significance of the observation did not occur to me until long afterward when I had seen it many times in other subjects.—Y. H.)

To the factor in the circulation thus indicated the term "veno-pressor mechanism" has been applied in previous papers from this laboratory (5), (11). We would suggest that it consists essentially in the chemical influence, either directly or through a local nervous mechanism (e.g., unineuronic reflexes), upon the caliber of the capillaries and especially of the small efferent vessels, the venules, exerted by the greater or less venosity of the blood in and flowing from the organs,—especially the skeletal muscles. The greater the activity of the muscles the more venous this blood. The efferent vessels are thus relaxed and the outflow from the capillaries into the venous system made easier. The volume of the venous return to the heart is increased, the venous pressure is raised and (within certain limits) the efficiency of the heart is increased (11). Thus the two extremes of high venous pressure on the one hand and abnormally low venous pressure on the other are induced respectively, the former by muscular exertion with great CO_2 production and the latter by acapnia and related conditions.

Cannon (12) has recently reported observations on wounded soldiers which suggest that in shock the decrease of venous return may be due to a narrowing of the capillaries and venules so that the red blood cells are accumulated in the periphery. A decrease of flow through the arm and a marked pallor of the skin have been observed here, both after forced breathing and ether hyperpnoea (6), (8).

In these statements we are not denying or questioning the results of a long line of investigators whose work has hitherto been accepted as demonstrating the vasomotor control of the venous return. In fact our own experiments given below show that the vasomotor nervous system exerts a marked influence upon venous pressure. Our view is merely that in addition to this influence there is a mechanism providing a direct peripheral chemical regulation of the venous return to the heart. In support of this view we offer a new and, we believe, crucial observation. Kaya and Starling found that when the lungs of a headless animal are ventilated with air containing a high percentage of CO_2, arterial pressure is not affected (13). We have verified their statement on this point, but we have to add to it a new fact, namely that in such an experiment *venous pressure undergoes a very marked increase.*

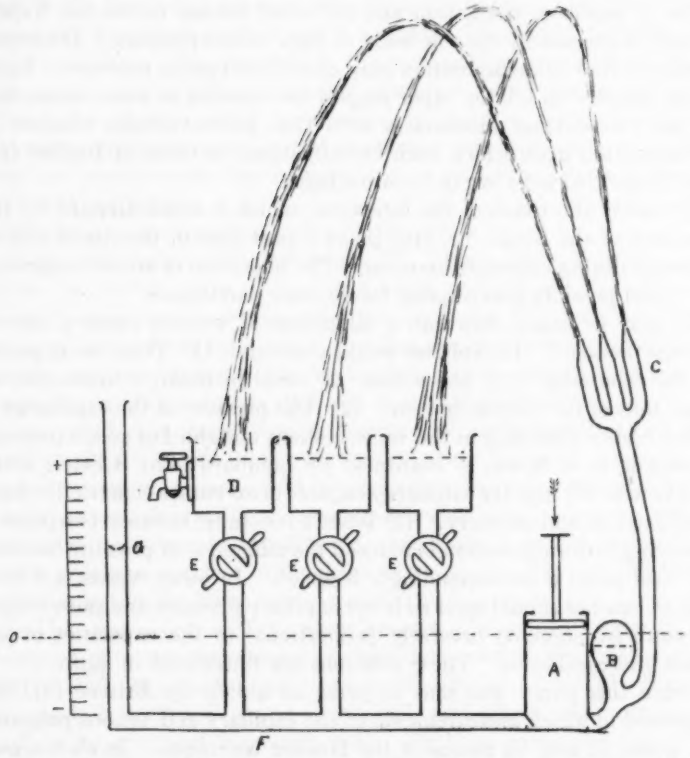

Fig. 1. Diagram to illustrate how a relaxation of venules may be expected to induce a rise of venous pressure. The system shown consists of a pump, *A*, an elastic chamber, *B*, and fine nozzles, *C*, corresponding to the arterioles controlled by the vasomotor nervous system. The jets of fluid after losing a part of the energy imparted by the pump fall into the reservoirs, *D*, representing the capillaries of the tissues and organs. Close below these reservoirs, on the tubes leading back to the pump, are cocks, *E*, which if partly closed will diminish the (venous) return to the pump and cause a lowered (venous) pressure in *F*. If the cocks are widely opened the pressure in the (venous) system, *F*, will rise so that the top of the column, *G*, will be nearly level with the surface of the fluid in the reservoirs. If the cocks are partially closed the column, *G*, will be lowered, and the efficiency of the pump correspondingly decreased.

We hasten to add also that we are not claiming acapnia as the only cause of capillary stagnation and failure of venous return nor hypercapnia as necessarily the sole cause of high venous pressure. Doubtless toxins or cold or inflammation may cause low venous pressure. Facts to be reported in a later paper suggest the identity or close connection of the veno-pressor mechanism with that neuro-vascular element in inflammation upon which such investigations as those of Bayliss (14) and Bruce (15) have begun to throw light.

Probably the tonus of the intestines, which is much affected by the venosity of the blood (6), (16) plays a part also in the rise of venous pressure during muscular exertion. The liberation of stored corpuscles (17), and possibly also osmotic forces, may participate.

It may be asked, how can a dilatation of venules cause a rise of venous pressure? In reply we would point out, (1) That the capacity of the capillaries, i. e., the volume of blood in them, is much greater than that of the venous system. (2) The pressure in the capillaries is much higher than that in the veins,—about one-third of aortic pressure according to v. Kries, as confirmed by Lombard (42). Cotton, Slade and Lewis (40) find the capillaries capable of exerting a constrictive force of at least 30 mm. mercury. (3) When a resistance between two parts of a moving hydraulic system is reduced the difference in pressure between the two parts is correspondingly lessened. In other words, a dilatation of venules should tend to lower capillary pressure and raise venous pressure by amounts inversely proportional to the capacities of the veins and capillaries. These relations are illustrated in figure 1.

Since this paper was sent to press an article by Briscoe (41) has appeared in which measurements of the capillary and venous presssure are given on men by means of the Hooker technique. In eleven normal men the average capillary pressure is found to be 23.5 cm. of water, and in women 22.2 cm. of water or 13.0 and 13.5 higher than venous pressure. In individuals suffering from so-called irritable heart a constriction of venules causing an increase of capillary pressure is described.

LITERATURE

The literature of this subject falls under three main heads: circulatory failure, nervous (i.e., vasomotor) control of the venous return, and the responsiveness of the finer blood vessels to chemical influences.

1. That dealing with the circulatory depression and failure following trauma, toxemia and related conditions, has its beginning in the classic

papers of Goltz (18). In these papers he demonstrated reflex inhibition of the heart and vasomotor nervous mechanism. He thus established the nature of syncope and his explanation stands, probably forever, unshaken. But as regards the circulatory depression of shock the greater part of all the immense literature which has accumulated since Goltz consists merely in continually repeated attempts to make the same explanation apply; and these attempts have been, year after year and investigator after investigator, more and more complete and indisputable failures.

The first to recognize this was Porter (39).

Seelig and Lyon (19), Henderson (5), Mann (20) and others have shown by direct evidence that the explanation based on vasomotor failure is inadequate. Seelig and Lyon, Malcolm (21), and Henderson have emphasized the fact that even when the circulation is failing the arteries are constricted,—just as Wiggers (7) showed in hemorrhage, and that the failure cannot therefore be a vaso-relaxation.

Out of all this mass of experiments and argumentation one fact (first recognized in this laboratory and supported initially against great opposition) seems to have won general acceptance, namely that, while the circulatory depression of syncope consists mainly in a decrease of peripheral resistance in the arteries, *that of shock on the contrary lies almost wholly in decrease of the venous return.* In the one the factor is vasomotor, in the other veno-pressor (12).

2. That vasomotor activity may increase the venous return to the heart and raise venous pressure has been demonstrated by the investigations of Mall (22), Bayliss and Starling (23), Burton-Opitz (24), Plumier (25), Stolnikow (26), Roy and Brown (27) and others, and most clearly of all by those of Carl Tigerstedt (28). We have repeated many of the experiments of these authors ourselves with essentially the results which they obtained; but on the basis of this evidence we are inclined to doubt whether even the most vigorous exertion of the nervous control of the vasomotor mechanism can induce directly the relatively enormous increase of venous flow requisite to maintain and even raise venous pressure when the heart is pumping blood out of the venous system at the rate it does during utmost physical exertion. The usual supposition is that, as Hooker (29) expresses it,

> A vasoconstriction occurs in the great splanchnic area, including the portal vein, which shunts the blood to the active muscles. There is as a consequence a venous plethora which expresses itself as a rise of venous pressure which continues through the period of activity.

Or as Starling (30) expresses it,

> If a man starts to run, his muscular movements pump more blood into the heart, so increasing the venous filling, while the central nervous system, by contracting the arteries of the abdomen, increases the peripheral resistance, raises the arterial pressure, and forces all the available blood through the active muscles.

We do not doubt the qualitative correctness of these statements although we do question whether they tell the whole story. The chemical influence upon the blood vessels exerted by the altered blood gases during exercise must, we think, be taken into account as an accessory to the muscular movements and nervous vasoconstrictions and dilatations in respect to the rise of venous pressure.

3. The responsiveness of the blood vessels to direct chemical stimulation was observed first in capillaries by Severini (31).

Gaskell (32) contributed well known and important experiments and imputed great importance to the fact that the chemical changes, especially acid production, going on in an organ during activity may directly bring about a dilatation of the blood vessels of this organ and so, without the intervention of the nervous system, regulate its own blood supply according to its own needs. Gaskell thought that in order to be of any effect upon the volume of blood flow into and through an organ this dilatation must occur in the smaller arteries and not merely in the capillaries. He seems not to have considered the, as it seems to us, even more probable dilatation of the small veins and the more ready outflow into the venous system under the influence of the chemical products of activity.

Bayliss (33) demonstrated that under constant pressure an increase of flow through an organ occurs when CO_2 or other acid is added to a perfusion fluid. He recognized that any acid stronger than carbonic merely liberates CO_2 from carbonates and is itself neutralized. Thus the effects of all acids are brought about through CO_2, although in last analysis the stimulus may depend on C_H.

Hooker (29), Schwarz and Lemberger (34) and v. Anrep (35) have contributed experiments showing with entire unanimity and conclusiveness the relaxing effect of CO_2 and other acids in high dilution on blood vessels while Adler (36) has shown this effect and with special distinctness the contracting effects of dilute alkali. The details of the observations of Adler and previously of Natus (37) in related lines are especially interesting.

Hooker's work was done in part to test the idea of a veno-pressor mechanism as suggested by Henderson. Hooker rejects the idea and Bayliss likewise sees little to support it. Hooker appears to have misunderstood Henderson as claiming that an increase of acidity constricts veins. The contention of the latter was, on the contrary, that a high content of CO_2 in the blood and a high venous pressure are concomitants, and that low CO_2 induces low venous pressure. If the conception of the circulation expressed in figure 1 is at all correct, it is a mechanically erroneous idea that to raise venous pressure there must be a constriction of veins, and that dilatation of veins causes lower venous pressure. Hooker and Bayliss seem to read "veno-pressor" as synonymous with "veno-constrictor." On the contrary, as we conceive the hydraulic relations a widening of the caliber of the venules, allowing readier outflow from the tissue reservoirs, should increase venous pressure; and a constriction of venules should decrease the venous return to the heart, and lower venous pressure by damming the blood in the capillaries (see fig. 1).

None of the investigators in this field seem to have suggested that the chemical influence of tissue metabolism must be far more completely exerted upon venules than upon arterioles. Gaskell thought that arterioles may be affected through the lymph. But the inner wall of these vessels is bathed by the fresh arterial blood in which the CO_2 content varies comparatively little. Furthermore in normal life the chief control of the arteries is not chemical but nervous and is exerted from the central nervous system. The walls of the venules on the contrary are exposed both from within and without to the influence of metabolites and especially to the full and widely varying venous tension of CO_2, H_2CO_3, and C_H.

The experiments reported below are a few abbreviated illustrations taken from a very large mass of work. They were all performed and reported in outline eight or ten years ago (2). Full publication has been delayed until now in the hope that we should find some form of presentation which would bring the facts of venous pressure under the prevalent vasomotor conceptions.

EXPERIMENTAL

The general plan of our research was to do two sets of experiments for comparison. In one, series *A*, the nervous vasomotor mechanism was excited or depressed by the classic methods—section and stimulation of the spinal cord, stimulation of the splanchnic nerves, stimulation of an afferent nerve and intravenous injection of epinephrin. In the other, series B, as nearly as possible only the purely peripheral chemical factor in the regulation of the circulation was brought into activity by

adding CO_2 to the oxygen with which the lungs were insufflated in a decapitated animal. In both series the arterial pressure was taken as the index of the activity of the vasomotor nerve endings. In both it is the amount of the effect obtained upon venous pressure which is the point of interest.

To make the line of thought clear, let us suppose that it could be shown that increased activity of the nervous mechanism (e.g., by epinephrin or splanchnic stimulation) caused a great rise of arterial pressure but no change of venous pressure. Suppose again that increase of CO_2 in the blood and tissues caused a marked rise of venous pressure accompanied by no change of arterial pressure. Then we might fairly differentiate two mechanisms; the one nervous-arterial, the other chemical-venous.

Actually of course the various parts of the circulation are too closely interdependent for such experimental results to be obtainable or even conceivable. Even if the nervous system had no direct control over veins, arterial changes must indirectly influence venous pressure, and even if arterioles were wholly insensitive to chemical influences, venous changes must exert some indirect effect upon arterial pressure. As both of these conditional clauses are contrary to fact the problem of experimental differentiation of the nervous-arterial and chemical-venous controls is one of extreme technical difficulty.

Logically all possibility of the participation in venous pressure changes by alterations of cardiac activity or by stimulation or depression of nerve centers direct or reflex should be experimentally excluded. Such rigor of demonstration appears however to be only partially obtainable.

Any cardio-inhibitory action raises venous pressure by decreasing the volume pumped out of the venous system and by slowing the general blood stream (25). The blood gases strongly influence the cardio-inhibitory center; therefore in most experiments both vagi were cut.

During ordinary asphyxia there is an enormous rise both of arterial and venous pressure even after section of the vagi. If the peripheral effects of CO_2 contribute to this rise, the veno-pressor element is obscured by the powerful central vasomotor influence of asphyxia (38). For demonstration of the veno-pressor factor it is essential that the well-known and powerful effect of the asphyxial chemical substances and deficiencies upon the vasomotor center in the medulla should be excluded. Therefore (except in certain cases) the spinal cord was exposed and cut just below the occiput in dogs, while in the cats used the head was removed by the method of Sherrington.

When the stump of the spinal cord is stimulated electrically to determine the effects of electrical excitation of the vasomotor mechanism upon the circulation all the skeletal muscles also are thrown into contraction and this contributes to the rise of pressure by an indefinable amount. Therefore the animals in which this experiment was tried were always curarized.

When the cord is electrically stimulated or when epinephrin (Parke, Davis & Company adrenalin) is introduced into a vein it is necessary to distinguish between the effect upon venous pressure induced directly and effects indirectly resulting from the slowing or overstrain of the heart. Vagus section and care as to dosage are the only, yet not wholly satisfactory, means of covering this point.

Fortunately for the purpose of our research the results of the first series of experiments indicate that the nervous control of the circulation is chiefly on the arterial side, while the results of the second series demonstrate that the peripheral chemical control is powerful in its influence upon venous pressure and scarcely perceptible in its relation to arterial pressure.

Series A. These experiments are controls showing the maximum extent of the vasomotor nervous influence upon venous pressure. They show that *a*, abolition of the influence of the vasomotor center in the medulla by section of the spinal cord causes no fall (but sometimes indirectly a rise) in venous pressure; *b*, stimulation of the stump of the spinal cord sufficient to cause a marked rise of arterial pressure has only a slight and apparently mainly indirect effect in raising venous pressure when skeletal muscular movements are excluded by means of curare; *c*, reflex rise of venous pressure on stimulation of an afferent nerve is not comparable in amount to the arterial rise; *d*, stimulation of the splanchnic nerves induces a rise of both arterial and venous pressure. But in none of these conditions do the changes in venous pressure exceed 35 mm. saline and they are seldom so much, although the alteration of vascular tonus as judged by changes of arterial pressure, was in many cases intense,—facts which contrast strikingly with the results of chemical stimulation to be shown later (series *B*).

Methods. When dogs were used the technique was usually as follows: morphine subcutaneously (0.010 to 0.015 gram per kilo body weight); half an hour later a little chloroform, then ether. Vagi cut. Arterial pressure recorded by a manometer connected with the carotid artery. Venous pressure taken in the central end of the jugular or femoral vein by the method described by Henderson and Barringer (11). Great

care was taken, and is necessary, to insure avoiding any obstruction in the vessel. Even a full bladder obstructs the femoral return. Only the results of quick inflows were recorded (cf. loc. cit. (11)). Measurements of venous pressure are in millimeters of saline solution. Zero pressure was taken as the level of the junction of the vessel into which the special venous cannula manometer was inserted, and its proximal vein, the subclavian or abdominal vena cava. The protocols state when curare was used. After two or three concordant measurements of arterial and venous pressure the spinal cord was cut between the atlas and occiput and the effects upon arterial and venous pressures were noted. When the cord was stimulated electrically the electrodes were inserted into the vertebral canal on each side of the cord to a distance of a few millimeters below the point of section. Artificial respiration, unless otherwise indicated, was maintained by means of a pump run by an electric motor, or by intratracheal insufflation of oxygen from a cylinder of compressed gas through a wash bottle.

In some experiments the vena cava was partly occluded as a device to exclude cardiac effects upon venous pressure and confine the evidence to peripheral changes.

In the experiments in which the splanchnics were stimulated the spinal cord and vagi were not cut.

When cats were the subjects of the experiment they were first lightly etherized, the vagi cut and the cord severed just below the occiput, or else the entire head was removed by the method of Sherrington.

Experiment A 1. Bull dog; 5 kilos. Venous pressure from jugular vein. Insufflation.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before section of spinal cord	110	30
20 seconds after section	80	50
1 minute later	60	50
2 minutes later	60	50

Result: On section of the cord venous pressure, instead of falling, rose slightly, owing probably either to the passage of a greater volume of blood through the relaxed arterioles into the venous system, or to less efficient heart action.

Experiment A 2. Dog; 9.5 kilos. Venous pressure from femoral. Insufflation. The vena cava was partly compressed by a ligature placed about it just below the diaphragm, not sufficient to occlude it entirely but merely so as to cause and maintain a high venous pressure distally. It was thought that under these conditions any change in the tonus of the veins would be made more evident.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
6 minutes before section of spinal cord	120	240
Immediately before section	115	240
1 minute after section	90	240
4 minutes after section	80	240

Result: With a slight obstruction (not occlusion) of the vena cava the femoral venous pressure was not affected by spinal section although arterial pressure fell 35 mm.

Experiment A3. Bull dog; 9 kilos. Venous pressure from femoral vein (unobstructed). Insufflation. The animal was fully curarized.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before section of cord	160	60
3 minutes after section	80	75
4 minutes after section	70	60
5 minutes after section	60	50
The spinal cord was then stimulated electrically for 2 minutes, i.e., through 8th minute		
6 minutes after section	80	60
7 minutes after section	90	60
8 minutes after section	100	60
12 minutes after section	55	55
Stimulation of cord repeated with stronger current and continued for 6 minutes (through 17th minute)		
13 minutes after section	95	65
15 minutes after section	125	75
17 minutes after section	150	60
35 minutes after section	70	60

Result: On section of the cord arterial pressure fell one-half while venous pressure rose slightly, then fell slightly. On moderate stimulation of the cord there was marked rise of arterial, but only a slight rise of venous pressure. On strong stimulation arterial pressure was raised from 55 to 150 while venous pressure rose only from 55 to 75, and failed to maintain even this elevation to the end of the stimulation period.

Experiment A4. Bull dog; 13 kilos. Venous pressure from jugular. Artificial respiration after section of cord.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury.	*mm. saline.*
Before section of cord	120	45
15 seconds after section of cord	60	40
30 seconds after section of cord	40	40
40 seconds after section of cord	35	45
1 minute after section of cord	35	40
7 minutes after section of cord	35	40
Cord stimulated electrically for 1 minute		
9 minutes after section of cord	120	45
11 minutes after section of cord	60	30
Cord stimulated for 2 minutes		
13 minutes after section of cord	100	45
14 minutes after section of cord	100	40
15 minutes after section of cord	80	30
16 minutes after section of cord	65	30
34 minutes after section of cord	30	35
Cord stimulated for 5 minutes, (through 40th) curarization had begun to wear off		
36 minutes after section of cord	105	70
37 minutes after section of cord	100	65
38 minutes after section of cord	100	60
39 minutes after section of cord	100	50
40 minutes after section of cord	100	40
41 minutes after section of cord	80	35
42 minutes after section of cord	70	30

Result: On section of spinal cord arterial pressure fell from 120 to 35 with a barely perceptible change of venous pressure. Stimulation raised arterial pressure greatly, venous pressure only slightly. After the curare had begun to wear off and slight muscular movements were induced by spinal stimulation, a marked but not a lasting effect on venous pressure was induced.

Experiment A5. Dog; 9 kilos. Venous pressure from jugular. Insufflation. Animal curarized. Spinal cord cut. Left sciatic nerve exposed and cut. Central end of nerve stimulated electrically.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before stimulation	70	25
Stimulation of sciatic for 6 minutes		
1st minute	80	30
2d minute	90	30
3d minute	100	30
4th minute	110	30
5th minute	120	30
6th minute	130	30

Result: After section of the spinal cord stimulation of the central end of the sciatic nerve caused arterial pressure to rise gradually from 70 to 130 mm. without a corresponding effect on venous pressure.

Experiment A6. Dog; 10 kilos. Morphin. Ether. Artificial respiration. Stimulation of right splanchnic in thorax.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before stimulation	120	30
Splanchnic stimulation	150	50
After stimulation	110	20

Result: Stimulation of the splanchnic caused a rise of both arterial and venous pressures.

Experiment A7. Dog; 12 kilos. Morphin, ether, artificial respiration. Right splanchnic nerve stimulated in thorax.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before stimulation	120	45
Splanchnic stimulation	150	55
Stimulation stopped	130	45
Stronger stimulation	160	55
Stimulation stopped	130	45

Result: Stimulation of the splanchnic nerve caused a rise of both arterial and venous pressure.

Experiment A8. Dog; 11.5 kilos. Morphin, ether. Natural breathing of air under a sufficient positive pressure to keep the lungs distended after the thorax was opened. A cardiometer was placed upon the ventricles of the heart and the volume curve recorded. From the heart rate and amplitude of beat the circulation rate has been calculated. The right splanchnic was cut and stimulated.

	HEART RATE BEATS PER MINUTE	CIRCULATION RATE	ARTERIAL PRESSURE	VENOUS PRESSURE
		cc. per minute	*mm. mercury*	*mm. saline*
Before section of splanchnic	90	1,980	140	70
After section of splanchnic	100	1,300	95	45
During stimulation of splanchnic	100	1,600	130	50
During stronger stimulation	85	1,745	165	60
After stopping stimulation	65	1,495	110	50

Result: Section of the right splanchnic caused a fall of both arterial and venous pressure, a less efficient diastolic filling of the heart and a consequent decrease of circulation rate. Stimulation of the splanchnic raised both pressures and increased the venous return as evidenced by augmentation of the circulation rate. For graphic records from this animal see Henderson and Barringer, op. cit. p. 360.

Experiment A9. Cat, decapitated. Insufflation. Quick injection of adrenalin, complete in 10 seconds.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before injection	95	50
30 seconds after	140	70
2 minutes after	80	50

Result: The arterial pressure rose 45 mm. and venous 20 mm.

Experiment A10. Cat, decapitated. Insufflation. Slow injection of adrenalin during 2 minutes.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before injection	20	5
20 seconds after starting injection	150	40
1 minute after starting injection	150	30
2 minutes after starting injection	150	10
2½ minutes after starting injection	140	5
5 minutes after starting injection	30	5

Result: Although venous pressure rose at first it fell again even during the injection nearly to its initial level, indicating that the vasomotor influence upon

venous pressure is not direct but is due to redistribution of blood. As soon as this redistribution is established venous pressure falls again even while a high arterial pressure is maintained.

Experiment A11. Cat, decapitated. Insufflation. Slow injection of adrenalin, during 4.5 minutes.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before injection	40	0
30 seconds after starting injection	150	20
1 minute after	150	20
2 minutes after	140	10
3 minutes after	130	5
4 minutes after	120	3
5 minutes after	100	0

Result: Although venous pressure rose at first it fell again even during the injection nearly to its initial level.

Series B. These experiments were designed to test the peripheral chemical vascular control, i.e., the veno-pressor mechanism. They were performed upon cats after decapitation, or section of vagi and spinal cord. The pulmonary ventilation was maintained by intratracheal insufflation with a jet of oxygen.

When the jet was enriched with 15 or 20 per cent of CO_2, venous pressure began after a few seconds to rise and continued to do so until levels of 60 or 70 mm. or even more were attained. When the CO_2 was shut off and the lungs ventilated rapidly with oxygen alone, venous pressure fell again gradually to its original level.

These changes of venous pressure are about 100 per cent greater than those obtained in the previous series of experiments. Their significance is clear in view of the fact that, while in the previous series arterial pressure was always greatly affected, in the effects of CO_2 arterial changes are minimal or absent. The only effect upon the arterial pressure curve was an increased amplitude of pulsation.

Venous pressure was measured from the jugular in all cases and from the femoral also in a few experiments in which the vena cava inferior was partially occluded.

Experiment B1. Cat, beheaded. Insufflation with oxygen, then oxygen plus 15 per cent CO_2, and finally oxygen alone.

	TIME	ARTERIAL PRESSURE	VENOUS PRESSURE IN JUGULAR
	minutes	*mm. mercury*	*mm. saline*
Oxygen alone	0	60	0
Oxygen + CO_2	1	60	5
Oxygen + CO_2	2	62	10
Oxygen + CO_2	2½	62	25
Oxygen + CO_2	3	64	35
Oxygen + CO_2	3½	64	40
Oxygen + CO_2	4	66	50
Oxygen + CO_2	4½	68	60
Oxygen + CO_2	5	70	70
Oxygen alone	5½	70	60
Oxygen alone	6	70	50
Oxygen alone	7	70	45
Oxygen alone	8	70	30
Oxygen alone	9	68	15
Oxygen alone	9½	68	10
Oxygen alone	10	66	5
Oxygen alone	11	66	0

Result: Under the influence of CO_2 venous pressure rose progressively during 5 minutes to a height of 70 mm. As the excess of CO_2 was ventilated out of the animal venous pressure fell again. The simultaneous changes of arterial pressure were 10 mm. upward and 4 mm. downward. The pressure record showed an increased amplitude of pulsation.

Experiment B2. Cat, beheaded. Insufflation with oxygen, then oxygen plus 15 per cent of CO_2, and finally oxygen alone.

	TIME	ARTERIAL PRESSURE	VENOUS PRESSURE
	minutes	*mm. mercury*	*mm. saline*
Oxygen alone	0	50	0
Oxygen + CO_2	1	50	0
Oxygen + CO_2	7	52	10
Oxygen + CO_2	16	58	30
Oxygen + CO_2	19	58	40
Oxygen + CO_2	20	60	45
Oxygen alone	22	60	60
Oxygen alone	23	60	50
Oxygen alone	24	60	45
Oxygen alone	25	60	35
Oxygen alone	26	60	25
Oxygen alone	27	55	10
Oxygen alone	28	55	0

Result: Under CO_2 venous pressure rose during 22 minutes from 0 to 60 mm. while arterial pressure rose only from 50 to 60. The amplitude of the pulsations in the arterial pressure record was markedly increased. Later during 6 minutes under oxygen alone venous pressure fell to its original level and arterial pressure sank slightly.

Experiment B3. Cat, vagi cut and spinal cord severed below occiput with dull chisel but head not removed. Insufflation with oxygen, then oxygen plus 20 per cent of CO_2, and finally oxygen alone. The vena cava inferior was partially (not completely) occluded and the pressure was determined in both the jugular and femoral veins.

	TIME	ARTERIAL	PRESSURES JUGULAR VEIN	FEMORAL VEIN
	minutes	*mm. mercury*	*mm. saline*	*mm. saline*
Oxygen alone	0	60	30	50
Oxygen + CO_2	1	70	20	65
Oxygen + CO_2	4	70	25	80
Oxygen + CO_2	5	70	35	85
Oxygen + CO_2	6	70	35	90
Oxygen alone	8	70	35	70
Oxygen alone	10	70	35	65
Oxygen alone	11	65	30	60
Oxygen alone	12	65	20	50
Oxygen alone	13	60	20	45
Oxygen alone	14	60	20	30

Result: The effects of CO_2 in this case were similar to those in the two preceding experiments but the fact that increased amplitude in the arterial pressure record occurred even with the vena cava damped suggests that this phenomenon is not due to increased filling of the heart, i.e., larger systolic discharges, but to more forcible contractions.

CONCLUSIONS

The general significance of the results of these two series of experiments appears to us to be as follows:

1. Procedures which strongly influence vasomotor innervation, e.g., spinal section, spinal stimulation, stimulation of an afferent nerve, splanchnic stimulation and intravenous injection of epinephrin, cause on the whole decidedly greater alterations of arterial than of venous pressure, and the alterations of venous pressure are often only momentary and therefore largely indirect and secondary to redistribution of blood.

2. In the beheaded cat increase of CO_2 in the blood (with ample oxygen), which has little or no effect upon arterial pressure other than an increased amplitude of pulse, causes an enormous rise of venous pressure. The pressure develops gradually as the CO_2 accumulates in the tissues and falls again gradually as the excess of CO_2 is ventilated out of the body.

3. These facts, taken with those quoted in the introductory part of this paper, indicate the existence of a veno-pressor mechanism distinct from the vasomotor nervous regulation and consisting in a peripheral chemical control, largely through variations in the CO_2 content of the venous blood, over the venous pressure and the volume of the venous return.

4. As venous pressure and the volume of the venous return are essential elements in the diastolic filling of the heart, and thus are factors in determining the volume of blood circulated in unit time, it appears probable that the veno-pressor mechanism may play a part in the increased circulation during muscular exertion. It thus assists in coordinating the volume of the blood stream with the energy expenditure and gaseous metabolism of the tissues.

5. As it is now generally admitted that in shock it is the decreased venous return which is the cause of the fall of arterial pressure, the relation of CO_2 to the veno-pressor mechanism affords an explanation of the mode by which acapnia induces circulatory failure.

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XII. THE INFLUENCE OF DRUGS ON INTESTINAL RHYTHMICITY

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It has been shown that, roughly speaking, the rate of rhythmic contraction in the small intestine varies inversely as the distance from the pylorus (1). The evidence in favor of the muscular origin of this rhythm seems now to be overwhelming. The theory of a neurogenous origin depended almost entirely upon the work of Magnus, which has recently been repeated and overthrown by Gunn and Underhill (2). Using a better technic, they obtained plexus-free strips of intestinal muscle that would contract rhythmically in Locke's solution. More recently Mr. Taylor and I (3) and Miss Starkweather and I (4) have presented evidence in support of the view that the local differences inrhythmicity are due to differences in the metabolism of the muscle in the different regions. This present work was undertaken in the hope that further light might be thrown upon this problem by a study of the ways in which various drugs influence the rate of contraction in the different regions.

TECHNIC

Excised segments of rabbit's intestine have been used. The animals were killed by a blow on the head and the abdomens opened immediately. Segments were cut from the first portion of the duodenum, from the upper jejunum, from the upper ileum, from the lower ileum and from the colon where it parallels the first portion of the duodenum. These pieces were washed out and kept in iced Locke's solution. Smaller segments, 2 to 3 cm. long, were cut as needed. These were attached to light heart-levers and suspended in a beaker containing 400 cc. of aerated Locke's solution kept at 38°C. When testing valuable drugs this large amount of fluid may seem wasteful but the results are much better than those obtained with small amounts. The segments beat more regularly, possibly on account of the greater dilution of their metabolites; and less care need be taken to avoid changes in temperature

when adding drugs. To insure prompt mixture the drugs were all added in liquid form. By directing the air-jet horizontally, a certain amount of circulation was kept up in the fluid. Most of the work was done with new segments which had not been tested previously with any drug. Ordinarily the segments that had been tested and washed were used for preliminary work in finding the dosage of the various drugs which would definitely influence the muscle but not paralyze it. After that a few crucial experiments would be done with new pieces.

Of the seventy-five drugs studied, a number are used commonly in medicine as laxatives and emetics, several are nerve depressants and anesthetics, some diminish or increase oxidation while others are poisons which attack various parts of the protein molecule. They have been arranged alphabetically for the sake of convenience. They may be divided into three classes: those which increased the rate, those which slowed it and those which had no definite effect with the doses used.

When possible, the rate was counted after the segments had been exposed to the drug for from ten to fifteen minutes. Sometimes this interval had to be shorter, as when the contractions ceased or became irregular. No attempt was made to estimate percentages of increase or decrease in the rates of the colonic segments after it was seen that they contracted too irregularly for accurate work. It is interesting, as showing the great difference between the muscle in small and large intestine that except in the case of a few drugs which made the colonic rhythm more regular and others which stopped it altogether, those which had pronounced effects on the rates of the small intestine had none on the rate of the large.

Drugs which increased the rate. The most pronounced effects were obtained with calcium chlorid, calcium lactate and benzene. The following tables show data obtained in typical experiments. In all these tables D stands for duodenum, J for jejunum, M for middle and I for ileum.

	WITH CALCIUM CHLORID									WITH CALCIUM LACTATE			WITH BENZENE		
	Before	After	Percentage	Before	After	Percentage	Before	After	Percentage	Before	After	Percentage	Before	After	Percentage
D.....	16.0	16.0	100	15.5	17.0	109	14.0	17.5	125	15.0	16.0	107	17.2	19.5	113
J.....	14.3	14.5	101	14.5	17.8	123	12.0	16.8	140	13.0	15.5	119	15.0	19.2	128
M.....	12.7	14.2	112	14.0	17.5	125	11.0	16.2	147	12.5	16.5	132	14.5	18.0	134
I.....	12.0	14.0	117	12.0	16.0	133	9.5	14.3	150	12.5	16.0	128	10.5	14.5	138

A similar graded effect was observed in some of the experiments with sodium and potassium hydrate.

	WITH SODIUM HYDRATE			WITH POTASSIUM HYDRATE		
	Before	After	Percentage	Before	After	Percentage
D.....	15.0	17.0	113	12.5	12.0	96
J.....	13.0	15.0	115	12.0	11.5	96
M.....	11.0	15.0	136	9.0	10.3	114
I.....	10.8	14.5	134	8.5	9.3	109

More often, however, the percentage of increase was about the same or else graded irregularly. Nicotin occasionally produced a graded increase in rate, as will be seen from the following figures.

	WITH NICOTIN					
	Before	After	Percentage	Before	After	Percentage
D.....	15.7	15.8	101	14.3	14.3	100
J.....	14.0	14.3	102	13.0	13.5	104
M.....	14.0	14.5	103	12.8	12.5	98
I.....	12.3	14.0	113	9.3	10.0	107

Ordinarily the results were more irregular. Ammonia gave one graded result among several atypical ones.

	WITH AMMONIA		
	Before	After	Percentage
D.....	15.6	16.0	102
J.....	10.8	12.0	111
M.....	10.2	11.5	113
I.....	8.2	11.6	141

A pronounced ungraded increase was observed several times with mercuric chlorid. Slight irregular increases were obtained at times with adrenalin, acetone, chloroform and ether. Ordinarily no changes in rate were observed with these drugs.

The striking thing, then, in a number of these experiments was the peculiar gradation in the percentage of increase. The possible significance of this finding will be taken up later in the discussion.

Drugs which slowed the rate. These may be divided first into two groups: those which had pronounced effects and those which had only slight effects.

Pronounced effects	*Slight effects*
Aloin	Alcohol (ethylic)
Alum $AlK(SO_4)_2 12H_2O$	Anilin oil
Antimonyl potassium tartrate	Apocodein hydrochlorid
Carbon dioxid	Apomorphin
Cascara	Barium chlorid
Chloral hydrate	Beta eucain
Digitalis	Chloretone
Ergot	Copper sulfate
Formaldehyd	Digitalin
Hydrochloric acid	Glucose
Ipecac	Hydrogen peroxid
Jalap	Lead acetate
Phenyl hydrazin	Magnesium chlorid
Potassium cyanid	Magnesium sulfate
Quinin bisulfate	Oxalic acid
Quinin hydrochlorid	Phenol
Quinin urea hydrochlorid	Pilocarpin
Senna	Potassium bromid
Sodium citrate	Potassium iodid
Sodium nitrite	Potassium permanganate
Sodium salicylate	Sodium fluorid
Zinc sulfate	Sodium phosphate (Na_2HPO_4)
	Sodium potassium tartrate
	Sodium sulfate

Thirteen of these substances will be discussed first as they all tended to slow the segments in a particular way. All but alcohol are from the group of strong depressants. They are:

Alcohol	Formaldehyd
Alum	Potassium cyanid
Antimonyl potassium tartrate	Quinin bisulfate
Carbon dioxid	Quinin hydrochlorid
Chloral hydrate	Quinin urea hydrochlorid
Digitalis	Sodium citrate
Ergot	

Following are some typical protocols:

	DIGITALIS			CHLORAL HYDRATE		
	Before	After	Percentage	Before	After	Percentage
D.....	12.4	12.5	100	16.8	11.0	66
J.....	11.5	10.0	87	11.4	6.0	53
M.....	8.7	6.5	74	10.2	5.0	49
I.....	8.2	7.5	91	9.2	5.0	55

	POTASSIUM CYANID					
	Before	After	Percentage	Before	After	Percentage
D.....	17.0	15.0	88	12.3	12.0	97
J.....	14.0	12.0	86	10.2	6.0	58
M.....	12.0	6.5	54	10.5	5.0	47
I.....	12.0	8.5	71	9.8	4.4	45

	CARBON DIOXID					
	Before	After	Percentage	Before	After	Percentage
D.....	13.9	11.3	81	14.0	13.2	94
J.....	13.7	11.0	80	13.5	11.5	85
M.....	11.7	6.4	55	11.5	8.2	71
I.....	10.6	5.4	51	10.0	9.0	90

Twenty-four such sets of percentages obtained with different drugs have been charted in figure 1. The ordinates represent percentages and the abscissae the four segments. It is apparent that there was always a gradation from segment D to segment M and then usually a rise to segment I. Occasionally the downward gradient was maintained as far as segment I. The percentage of I never rose as high as that of D.

The other drugs in the group of marked depressants which did not show this type of gradation are, with two exceptions, resinous vegetable extracts: aloin, cascara, ipecac, jalap and senna. With these, and with all but one (alcohol) of the mild depressants in group 2, the rates were depressed either irregularly or about equally.

Substances which did not affect the rate.

Acetone. Occasionally seemed to increase the rate
Adrenalin. Occasionally seemed to increase the rate
Atropin
Bile
Chloroform. Occasionally seemed to increase the rate
Cocain. Occasionally seemed to increase the rate
Codein. Occasionally seemed to decrease the rate
Eserin
Ether. Occasionally seemed to increase the rate
Glycerin
Lithium carbonate
Morphin sulfate
Novocain
Picric acid
Pitu trin
Potassium chlorid
Sodium bicarbonate
Sodium chlorid
Strychnin sulfate
Urea
Urethane.

It is possible that if larger doses had been used some of these drugs might have produced more definite effects. Some of them, such as chloroform and ether, could not be used in larger concentration on account of their comparative insolubility in water.

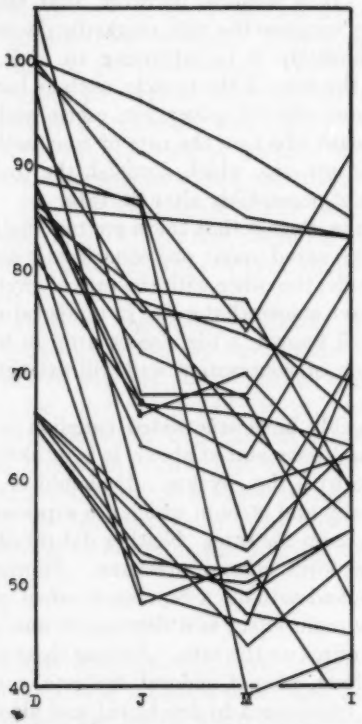

Fig. 1. Shows the gradation in the percentage of slowing in different regions. The ordinates represent percentages of the original rate before the drug was added; the abscissae represent the four regions from which segments were taken.

DISCUSSION

At first sight there seems little in common among the drugs which increased the rate. Ammonia and the hydrates of potassium and sodium may owe their effects to their alkalinity. Sodium bicarbonate, however, had no effect on the rate although it had definite effects on tone and amplitude of contraction. Hydrochloric acid and CO_2 slowed

the segments markedly but oxalic acid had little effect on the rate and picric acid had none. To be sure, the toxic effects of the last two acids were so marked that, in the small doses used, the acid factor may have been slight. Calcium salts are known to favor rhythmic activity in the heart muscle. It is strange, however, that they should, at the same time that they increase the rate, markedly decrease the amplitude of contraction. Similarly it is surprising to find that drugs which markedly increase the tone of the muscle, such as barium chlorid, copper sulfate, potassium chlorid, pilocarpin, eserin and pituitrin have, if anything, a depressant effect on the rate of contraction. Again, adrenalin, urethane and atropin, which diminish the tone and amplitude of contraction, do not perceptibly alter the rate.

These observations suggest that there are two distinct phases of the muscular metabolism acted upon: one concerned with the maintenance of tone and amplitude, the other with the rate of rhythmic contraction. Taylor and I (3) have shown that a rise in temperature will affect both together. As is well known, a big rise or drop in tone will generally affect the amplitude of contraction and will extinguish the rhythmic waves.

Nicotin may possibly have stimulated ganglion cells in Auerbach's plexus, although, as I have stated above, it is unlikely that this plexus has anything to do with the rhythm. It should be noted in this connection that adrenalin and atropin which are supposed to act on nerve endings had no effect on the rate. Neither did pituitrin, cocain, novocain, morphin, chloroform, ether or urethane. Pilocarpin, which stimulates nerve endings, had actually a depressant effect on the rate. Some of the drugs whose main effect is a depressant one on nervous tissue did have a slowing effect on the rate. Among these may be mentioned betaeucain, chloretone, phenol, chloral hydrate, magnesium sulfate, potassium bromid, quininurea hydrochlorid and alcohol. It must be remembered, however, that most if not all of these drugs have, besides their marked and well known effects on nerves, a more or less toxic action on muscle and on protoplasm in general. Those who may feel inclined to use these results in arguments for or against the neurogenous origin of the rhythm should be deterred not only by the contradictions pointed out above but also by the many contradictions in recent writings; contradictions which show that our ideas about the pharmacology of nerve endings must be revised. Some of these difficulties in the way of accepting the old ideas are well brought out by Cushny (5). It is not surprising that protoplasmic poisons such as potassium cyanid,

formaldehyd, phenylhydrazin, anilin, oxalic acid and potassium permanganate should have slowed the rate. Others, however, such as picric acid, had no effect; while mercuric chlorid and ammonia produced an increase in rate.

The gradations in the percentage of increase and decrease with various drugs are of considerable interest. They suggest a greater stability of the rhythm in the duodenum. The tendency to contract rhythmically is so much stronger in the upper end of the tract that it may, perhaps, overcome a depressant which is powerful enough to slow the ileum (6). The greater increase in the rate of the ileum after the addition of calcium salts suggests that the duodenal rate is already nearly maximal. The fact that some of the drugs produced graded increases or decreases while others had ungraded effects suggests that the two groups act in different ways or on different parts of the contractile protoplasm.

It is of interest that the vegetable cathartics, aloin, cascara, jalap and senna all produced a marked decrease in the rate of contraction. This effect could not be ascribed to the alcohol introduced with them as the amount was too small to have any effect. Another thing which deserves mention is the fact that digitalis had a pronounced slowing effect on the excised intestinal muscle just as it has on the heart (7). That CO_2 has a particular effect on the rate aside from its asphyxiating action is shown by the fact that no slowing appeared even when the segments were allowed to contract for long periods of time without aeration of the fluid.

SUMMARY

Sets of five segments from different parts of the bowel were studied under identical conditions in a beaker of aerated Locke's solution.

Of the seventy-five drugs tested, eight increased the rate, forty-six slowed it and twenty-one had no effect.

Calcium chlorid, calcium lactate, benzene, nicotin, ammonia, sodium hydrate, potassium hydrate and mercuric chlorid increased the rate.

Marked slowing was produced by alum, antimonyl potassium tartrate, carbon dioxid, cascara, chloral hydrate, digitalis, ergot, formaldehyd, hydrochloric acid, ipecac, jalap, phenylhydrazin, potassium cyanid, quinin salts, senna, sodium citrate and sodium nitrite.

With a number of the drugs the rate of the ileal segment was more affected than that of the duodenal segment; and there was a gradation in the percentage of increase or decrease from one end of the bowel to

the other. This suggests that the rate in the upper part of the gut is more stable and perhaps more nearly maximal than it is in the ileum.

Drugs which increase or decrease the tone and amplitude of contraction do not necessarily affect the rate. Thus, pilocarpin and barium chlorid which increased the tone, decreased the rate. Calcium salts which increased the rate diminished the amplitude of contraction. This suggests two phases of muscular metabolism acted upon: one concerned with tone and amplitude, the other with the rate.

Digitalis slows the intestinal contractions much as it slows those of the heart.

Excepting those cases in which the colonic rhythm was stopped entirely, it was practically unaffected by the drugs which caused marked changes in the rate of the small intestine.

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XIII. THE MOTOR FUNCTIONS OF THE CECUM

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It has been shown in recent papers that there are gradients of rhythmicity, irritability, latent period, CO_2 production and catalase content in the small intestine; gradients which we believe determine the direction of peristalsis (1). It occurred to us that there might be similar gradients in the muscular wall of the cecum, determining the direction of peristalsis in that organ. Some provision for the maintenance of orderly contractions would seem necessary, particularly in those birds which have ceca as long as the small intestine itself.

While studying a large number of (anesthetized) rabbits with their abdomens opened under salt solution, Alvarez (2) had an opportunity of watching the movements of the cecum. Except in animals with diarrhoea, this organ showed only occasional contractions at long intervals. The waves generally appeared near the apex, ran toward the base and then back again. Such movements ordinarily took place when a peristaltic rush forced material from the ileum through the ileocecal sphincter. Some of this material would apparently go into the cecum and some into the upper colon. Other observers have described similar movements. Meltzer and Auer (3), who watched the cecum through the shaved abdominal wall in rabbits, saw waves about once a minute. Katsch and Borchers (4), who studied the organ through a glass window in the abdominal wall, found it quiet for hours in some animals and more active in others. Elliott and Barclay Smith (5) say that in the guinea pig food from the ileum goes directly into the cecum. It is then passed backwards and forwards for awhile between cecum and colon. By giving the rabbits a definite number of small glass beads, which could be identified at autopsy several days later, they showed that the cecum retains its contents for many days. Swirski (6) showed that it would empty itself entirely after several days of absolute starvation. Basler (7) found that whereas in rats and cats there is almost

no churning of the cecal contents, in rabbits and guinea pigs the material is pretty well mixed.

Rhythmicity of excised muscle. We first looked for differences in rhythmicity in strips of muscle excised from five regions along the rabbit's cecum. The animals were killed by a blow on the head and

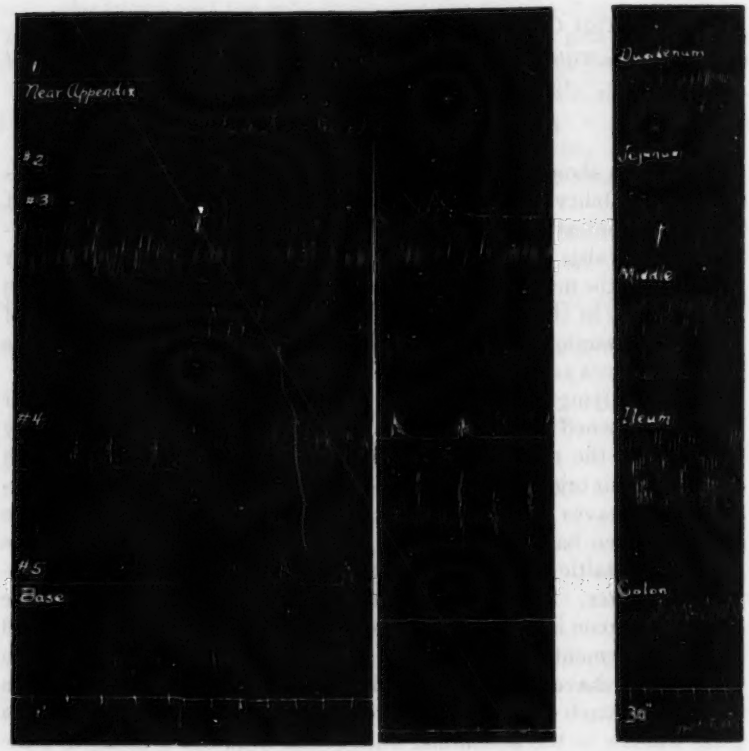

Fig. 1. Tracings from different parts of the rabbit's cecum. On the right, for comparison, tracings from different parts of small intestine and colon.

bits of muscle with mucous membrane attached were cut immediately. For the most part longitudinal strips were used. These pieces, about 1 cm. wide and 3 cm. long, were attached to light heart levers and suspended in a beaker containing 400 cc. of aerated Locke's solution at 38°C. All five of the segments beat poorly, less regularly even than

did the segments of colon which, as we have shown in a previous paper (8), have very little rhythmicity as compared with segments of small intestine. We could not make out much difference in the rhythmicity or in the shape of the contraction curves in different regions. Characteristic tracings are shown in figure 1. At intervals there were tonus waves upon which were superimposed a short series of contractions with a rate of from 8 to 16 per minute. After a while the tonus waves sometimes disappeared, leaving a fairly regular curve with perhaps 5 waves per minute. No difference was observed between the contractions of the longitudinal and of the circular strips. Segments of guinea pig's cecum showed even less tendency to contract rhythmically than did those from the rabbit; and no records of any value were obtained.

A glance at the records from cecum, small intestine and colon in figure 1 will show what a difference there must be in the neuromuscular apparatus in the three regions.

Irritability, latent period and form of contraction curve. Strips of muscle similar to those used in the experiments just described were placed in a warm, moist chamber (temperature 26° to 28°) and stimulated with a strong faradic current. A Harvard inductorium was used with the secondary coil at 0. The source of current was a battery giving 22 amperes at 1.5 volts. Even with this strong current the muscle responded very poorly and after a long latent period. The amplitude was so small and the rise from the base line so gradual that the latent periods could not be measured with any degree of accuracy except perhaps in the case of the strip removed from a point three-quarters of the way from the base of the appendix to the ceco-colonic junction. In the four rabbits studied this segment, number 3, always contracted well with an amplitude, on the tracings, of from 3 to 4 cm. The other segments raised the writing-point only from 0.2 to 1.2 cm. The lever magnified about four times. The following data are only approximately correct and would not be presented were it not that the gradation is so similar in all.

Latent periods.

Number 1 (near tip)	0.7	0.6	1.0
Number 2	0.7	0.5	0.5
Number 3	0.5	0.4	0.3
Number 4 (near base)	0.6	0.6	0.7

The figures represent seconds. There was no doubt about the comparatively good results with strip 3 and the poor results with strip 1.

The highest point on the contraction curve was reached ordinarily after 15 seconds in all the strips. Complete relaxation followed in from 2 to 5 minutes.

Catalase estimations. In a preceding paper (1) we have shown that the curve of catalase content of muscle from different regions of the small intestine follows closely the curves of rhythmicity and of CO_2 production. This parallelism was so marked as to make us feel that the catalase content of a tissue is a fairly reliable index to its metabolic activity. When dealing with regions like the cecum where one cannot learn much about the rhythmicity, where the latent period is hard to measure and where a number of errors can destroy the value of CO_2 estimations, it is a pleasure to have so simple and apparently so dependable a way of getting at the metabolic gradient.

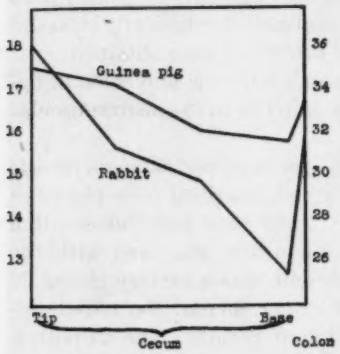

Fig. 2. Ordinates represent average values of catalase in figures of oxygen liberated in fifteen minutes. Those on the left are for the rabbit, those on the right for the guinea-pig. Abscissae represent regions along the cecum and in the colon opposite the cecum.

The method employed was the same as that described in the previous paper (1). The hydrogen peroxid was neutralized before using. All the five estimations were made at the same time under identical conditions of temperature, barometric pressure and shaking. Rabbits and guinea pigs were used. The first four strips were taken from the cecum; no. 5 was from the first portion of the colon opposite the mouth of the cecum. As the guinea pig has no appendix, the first strip was taken next to the tip. In the rabbit the first strip was taken near the base of the appendix. The mucous membrane was scraped off, and the muscle was weighed and ground in a mortar. The following figures represent cubic centimeters of O_2 at atmospheric pressure, liberated in 15 minutes by the catalase in 0.3 gm. of muscle. As the gradation in any one set is the important thing, we have not reduced the data to a common temperature and barometric pressure.

Rabbit

									Average
1. Near tip	16.5	13.5	12.4	23.2	21.2	31.0	15.2	11.8	18.1
2.	15.5	13.3	10.2	19.2	17.0	26.3	14.3	9.9	15.7
3.	15.5	10.4	11.2	14.1	16.5	28.8	12.7	9.5	14.9
4. Near base	11.6	10.3	11.2	8.1	13.0	22.5	14.2	8.7	12.5
5. Colon	15.0	14.1	11.7	14.6	18.5	26.7	16.3	9.4	15.8

Guinea pig

										Average
1. Near tip.	36.0	28.7	40.2	23.5	29.4	30.8	37.6	38.6	45.1	43.5	35.3
2.	27.4	23.0	49.2	22.0	26.8	27.7	34.0	34.8	47.4	55.6	34.8
3.	26.1	24.0	37.8	21.0	26.4	27.8	30.8	30.6	43.7	53.7	32.2
4. Near base	26.0	26.2	39.5	21.8	27.5	27.8	33.3	25.5	37.5	50.3	31.5
5. Colon	26.8	25.4	35.7	18.5	26.0	25.6	33.5	31.5	52.5	58.7	33.4

From these results it appears that there is a downward gradient from the tip to the base of the cecum in rabbits and guinea pigs (see fig. 2). From the base the gradient is upwards to the colon. In the guinea pig the difference between the catalase in any two adjacent segments is so near the limit of accuracy of the technic that it is not surprising that, in a number of the animals, we failed to show the usual gradation.

DISCUSSION

We have here to deal with a food reservoir which, in order to retain its contents, must contract but seldom and must not be very responsive to happenings in other parts of the digestive tract. As we should expect, then, we find it lined by muscle of low rhythmicity and low irritability. Judging by its catalase content, its metabolic activity is low. In the rabbit the figures range from 18 at the tip to 12 at the base. In the small intestine of the same animal the figures range from 38.5 in the duodenum to 21.9 in the ileum. It is interesting, in view of the much higher catalase values in the guinea pig, that Elliott and Smith state that the cecum in that animal is much more active than it is in the rabbit.

If the catalase determinations are indices of metabolic activity in the different regions and if, as we believe, peristalsis follows metabolic gradients, then the gradient from the colon to the cecum will explain the filling of the pouch, and the gradient from tip to base will explain the direction of the waves that empty it. In the stomach, small intestine and colon, as far as we have studied them, the gradient of latent period agrees quite closely with the gradients of rhythmicity, catalase content and CO_2 production. There are some exceptions, however. In

the intact frog the cardia is much more irritable than the antrum but when strips are excised the cardia is less sensitive than the antrum and its latent period is much longer (8). It seemed pretty clear, from a number of other observations, that the poor reactions obtained with the cardiac strips were due to their great susceptibility to the trauma of excision. The muscle in the antrum, on the other hand, was quite immune to trauma. Similar differences have been observed in all our work with segments of duodenum and ileum. It may easily be, then, that the failure of the latent periods to follow the expected gradient is due to a greater susceptibility to trauma at the tip, where the waves take their origin. It may be, also, that the powerful reactions of strip 3 are due to the presence of a stronger and more efficient muscle at this point, where the large bulk of the contents demands it.

The observations recorded at this time bring added support to the thesis proposed in the previous paper: that the peculiarities of function in the different parts of the digestive tract and the direction of peristalsis in the muscle are to be ascribed to local peculiarities in the muscle (and probably to a much less degree to peculiarities in the nerve net) in the different regions. The low irritability of the cecum may be ascribed partly to the poor development of Auerbach's plexus in this region. Gerlach (9) has shown that the meshes of the nerve net are very large as compared even with those in the ileum. The individual muscle fibers may thus be poorly connected one with the other.

SUMMARY

Excised strips of muscle from the cecum in rabbits and guinea pigs show little tendency to contract rhythmically in oxygenated Locke's solution. The records obtained are different from those traced by segments of small or large intestine. The irritability of the strips is low and the latent periods are long. The low catalase content of the muscle suggests also that its metabolism is sluggish. These peculiarities probably account for the retention of food in this organ for long periods of time.

There is a gradient in the catalase content of the cecal musculature from tip to base. This gradient probably corresponds to a metabolic gradient which determines the direction of peristalsis when waves do appear.

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HISTOLOGICAL STUDY OF FAT CONTAINED IN THE MUCOSA OF THE ALIMENTARY TRACT OF MODERATELY STARVED CATS

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INTRODUCTION

The comparative ease with which fat droplets can be demonstrated in the tissues by means of the various specific stains has greatly facilitated microscopical examination and furnished a stimulus for histological investigation along this line. The present study deals with the presence of these droplets or granules in the mucosa and in the gastric, intestinal and duodenal glands of animals after feeding had been suspended for 24 to 28 hours; i.e., the normal fat constituent, independent of fat absorption.

Many interesting investigations have been made from time to time in an effort to trace the passage of the fat content of the food from the lumen of the gut, through the mucosa and into the vessels of the alimentary wall. With this histological picture the reader is doubtless familiar. So far as I know, however, no previous investigator has interested himself primarily with what may be conveniently termed the normal fat droplets contained in the alimentary tissues, although reference has been made to these or similar bodies by a number of authors.

Eimer ('67), in considering the phenomenon of fat absorption in the small intestine, reported that in some animals one may find individual droplets in the cylindrical cells several days after fat absorption has taken place. He used Schultze's iodine serum for isolating them.

Greene and Skaer ('13) in their histological studies of fat absorption in the mammalian stomach, made the following observations: (1) Traces of fat were found in animals that had been kept without food for 20 to 24 hours, the droplets being located usually at the basal portion of the superficial cells of the mucosa. (2) In puppies and kittens that had not been fed the tissues of the stomach showed the presence of granules,

extremely small but possessing the characteristics of fat granules both in appearance and distribution. (3) It was difficult to cause the entire disappearance of fat from the gastric gland-cells by prolonged fasting and occasionally, after moderate to long fasting, the quantity of fat in these cells was even increased. This phenomenon they atrributed to mobilization of body fat. (4) There was a disappearance of fat from the cells during the early stages of fat absorption, which was thought to be due to an increased production of lipase with resulting hydrolization and solution of the fat droplets. In this condition the fat content was not demonstrable by the staining methods used. These investigators made their observations from frozen sections of material that had been fixed in 10 per cent formalin and stained with an alcoholic-alkaline solution of scarlet red.

Mendel and Baumann ('15), in considering the question of fat absorption from the mammalian stomach, resorted to histological methods as a check upon physiological experiments. Cats and dogs were experimented with and pieces selected from the pyloric and fundic regions of the stomach, fixed in Fleming's solution and stained with haematoxylin; or, in a few instances, pieces were fixed in formalin and frozen sections were made and stained with Sudan III. In these experiments there was surgical interference. The authors found few or no fat globules in the mucosa of animals that had been starved for 24 hours. Moreover, in a considerable number of sections practically no more fat was demonstrated in fed animals than in controls, and even when pieces of the stomach had been selected from regions most favorable for the absorption of fat, sections practically devoid of fat were obtained. The distribution was not uniform.

Very interesting findings have been reported by Gay and Southard, ('07) who studied the phenomenon of anaphylaxis in guinea pigs. In seeking pathological lesions they observed a striking picture of fatty change in the walls of the stomach. They examined material fixed with formaldehyde after the Marchi method, supplementing it to a limited extent by examination of fresh tissue in glacial acetic acid and by examination with Scharlach R. In the majority of the cases the animals died within an hour after receiving the second dose of serum. The authors assert that there is no reason to believe that fat is normally present in the gastric mucosa and that the results of their own investigations indicate that in the guinea pig the stomach under normal conditions is free from fat. Moreover the anaphylactic method failed to disclose the presence of fat in the stomach wall. The toxic material,

however, showed characteristic fatty changes in various foci while adjacent areas of tissue contained no fat whatever. The controls and anaphylactic stomachs were negative for fat by the Marchi method. The focality of the lesions, the associated congestion and at times the erosion of the surface, were considered to be wholly convincing evidence of the pathological character of the fat shown. Gross hemorrhagic lesions of the stomach were found in a majority of the cases and were always associated with fat in the gastric epithelium and in the endothelium of the neighboring vessels. The authors were inclined to regard the hemorrhages as dependent upon rupture of vessels weakened by fatty change, and the fat of the gastric epithelium as often of independent origin and not necessarily associated with vascular lesions. The fat pictures are illustrated by plates.

EXPERIMENTAL PROCEDURE

From a number of cats experimented with six were chosen from which the observations embodied herein were made. The records kept of these animals are more complete than in my earlier experiments and some of the more obvious technical errors have been avoided. All of the animals were full grown, apparently in good, healthy condition, and the weights of five of them ranged from 3 to 6½ pounds. All were starved for 24 to 28 hours before death. Three of the animals were killed by chloroform, two by a blow on the head and one by gas. Autopsies were performed immediately and the contents of the alimentary tract examined. The lacteals were grossly inspected in two of the cases. As quickly as possible portions from the walls of the stomach, duodenum and lower part of the small intestine were removed from each of the animals and placed in the fixing fluid. The portion of the stomach wall was selected from a segment between 2 and 3 cm. or 2 and 5 cm. above the pylorus. The portion taken from the wall of the duodenum comprised the segment extending from the pylorus to a point 2 or 3 cm. below the pyloric valve. In the later experiments the section taken from the lower part of the small intestine occupied the segment between 32 and 30 cm. above the valve of the colon.

These pieces, taken from the various divisions of the alimentary tract, were fixed in 20 per cent formalin.[1] Four hours were found to be sufficient for fixation but for the sake of convenience this period in some

[1] The formalin was freshly distilled and diluted with normal salt solution to make up the required strength. (Mann, G.: Physiological histology, 1902, 88).

instances was extended. The shortest possible time, however, is preferable (Bullard, '12–'13). After the tissues were taken from the fixing fluid they were washed thoroughly to remove the formalin. Frozen sections were made with the freezing microtome set at 5–15μ. The sections from each division of the digestive tract were divided into two groups. Those of one group were passed through the grades of alcohol (30, 50, 60, 70, 80, 90, 95 per cent) into absolute alcohol or chloroform. Here they were permitted to remain until all the soluble lipoids were removed. These were used as a control for the other specimens. The sections in the other group, which were not treated with absolute alcohol, were passed through 30, 40 and 60 per cent alcohol and then stained with a mixed alcoholic-alkaline solution of Scharlach R and Sudan III.[2]

The staining was done in covered vessels. The granules were found to be satisfactorily colored after the sections had been in the stain for 5 to 10 minutes. Precaution was taken to hurry the washing and hydrating process by running the sections quickly through 50, 60 and 30 per cent alcohol into distilled water, in order to avoid any destaining effect that the alcohols may have upon the fat droplets. It seems best not to allow the sections to remain in the 60 and 50 per cent alcohols longer than 5 to 10 seconds, while in the 30 per cent alcohol a much longer time may be taken without harm. After being removed from the distilled water the sections were lightly counterstained with Erlich's haematoxylin. The mounting was done in either glycerine or sugar.[3] The sections that had been treated with absolute alcohol were first hydrated to 60 per cent alcohol and then stained in a similar manner. Photomicrographs were made from selected sections, a green filter being used. The thickness of the sections made the necessary focusing very difficult.

Gross findings. The autopsies, which were done immediately after death, showed in each case that the stomach and small intestine were empty and collapsed while the large bowel was engorged with fecal matter which at times crumbled when removed. In the duodenal

[2] This stain is composed of the folllwing ingredients: Scharlach R, ½ gram, Sudan III, ½ gram, sodium hydroxide, 2 grams, alcohol 70 per cent, 100 cc. (suggested by Dr. H. H. Bullard).

[3] The solution of sugar in which the specimens were mounted was prepared as follows: 70 cc. of distilled water, 15 cc. of a saturated solution of glucose and 5 cc. of glycerine were mixed together. Then 5 cc. of spirits of camphor were added and the mixture filtered.

portion of the gut bile-colored mucus could be observed and a trace of perhaps indigestible matter was encountered in the small intestine. There were no evidences of fat absorption when the lacteals were inspected.

Microscopical findings. Microscopical examination of sections from the various divisions of the alimentary tract disclosed three types of red objects: (1) Stained fat globules of the submucosa; (2) precipitate; (3) colored fat droplets of the mucosa, and of the gastric, intestinal and duodenal glands.

1. Fat globules of the submucosa: It is not possible to confuse the familiar picture of fat globules normally present in the submucosa with the two other types of stained objects. Apart from their large size and globular form, their location in the submucosa would identify them.

2. Precipitate: Carrying out the directions for staining the fat constituents of the tissue resulted in the production of a precipitate. This might easily have been avoided had the stained sections been washed more thoroughly in 60 per cent alcohol. However, this was deemed unwise because of the risk necessarily involved in subjecting the fat granules to the destaining action of the alcohol. Fortunately, the precipitate possesses certain characteristics that make its differentiation from the fat droplets not only possible but usually quite easy. Three chief points should be borne in mind: (1) The precipitated particles have no definite distribution while the fat granules have; (2) the precipitate may be observed on both surfaces of a section when the microscope is focussed at these two levels, which is not true of the fat granules; (3) it can be distinguished from fat granules by its size and form. Rod- and needle-shaped bodies are sometimes abundant and when once noted are readily distinguishable thereafter. Unfortunately however, these precipitate bodies vary in size and shape, assuming forms that may be confused with the fat granules in the tissues. In these studies such confusion was obviated by always examining two series of sections. It will be recalled that one series was treated with absolute alcohol while the other, from the same divisions of the digestive tract, was not subjected to this treatment. This was done in order that a more definite differentiation between the precipitate and the fat granules might be made. In the sections treated with absolute alcohol it was possible to dissolve out the fat granules and thus destroy the fat picture. Therefore, since all the sections received the same treatment it was possible to examine the two corresponding series—

one containing fat granules and precipitate, the other, only precipitate. This afforded a means of comparison and was always done in instances where there could be any doubt. After a little experience with the precipitate the confusion at first encountered was practically nil. Aside from distinguishing it from the fat droplets, the precipitate was, of course, of no interest.

3. Fat droplets of the mucous lining, and of the gastric, intestinal and duodenal glands: Whereas a large proportion of the precipitated

Fig. 1. A photomicrograph of a frozen section of the stomach of a cat, stained with a mixture of Scharlach R and Sudan III. The droplets of stained fat show as dark spots in the bases of the epithelial cells over the surface. Note that they are absent from the cells of the depth of the foveolae. The dark needles are crystals of the stain. The cat was starved for 24 hours and killed with chloroform. × about 293.

particles are rod-shaped, the normal fat in the mucosa and adjoining glands appears in the form of more or less spherical droplets. These vary considerably in size and are seen collectively only when examined with the low power of the ordinary laboratory microscope. A conception of their size in comparison with that of the cells in which they are found, as well as their abundance in these cells, may be obtained by referring to the photomicrographs, which were made from the various

divisions of the alimentary tract (see figs. 1 to 6). The amount of fat varied considerably in the different animals. In some unexpectedly large quantities were present while in others the amount was only moderate. In one instance only a trace of fat was found. In general the largest amount was observed in the duodenum or small intestine. By the method employed the fat granules were, of course, stained red and when the microscope was focussed carefully their centers became somewhat yellow. There were no marked differences in the color of the

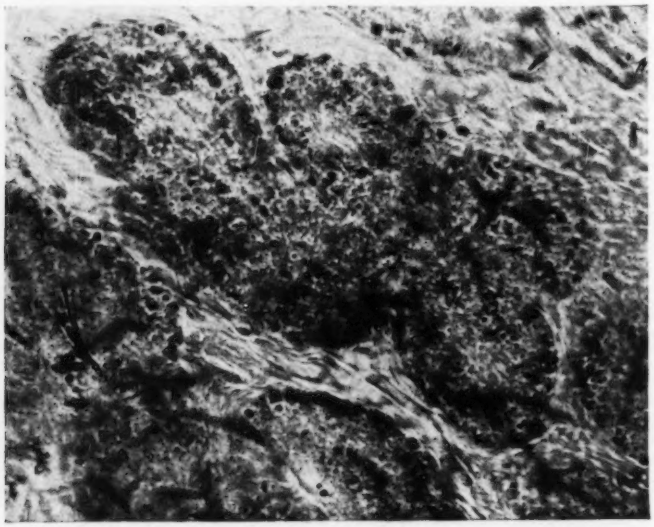

Fig. 2. A photomicrograph of a section of the stomach of a cat showing droplets of fat in the deeper portions or fundus of the gastric glands. The cat was starved about 27 hours and killed by a blow on the head. × about 293.

droplets in different divisions of the alimentary tract. The fat granules were extracted from those sections that had been treated with absolute alcohol.

Fat droplets were present in the gastric mucosa of all the animals, the quantity varying from a relatively large amount to a mere trace. These granules were found in the epithelial cells bordering the mouths of the gastric foveolae (fig. 1). In one of the animals the fat was demonstrated more or less continuously throughout these cells while in

others it occupied groups of cells. This focal arrangement recalls the findings of Gay and Southard ('07) referred to above. In the surface cells themselves the granules may appear on both sides of the nuclear zone and one is at a loss at times to determine whether or not they extend into the tunica propria or stroma of the mucosa. They have a

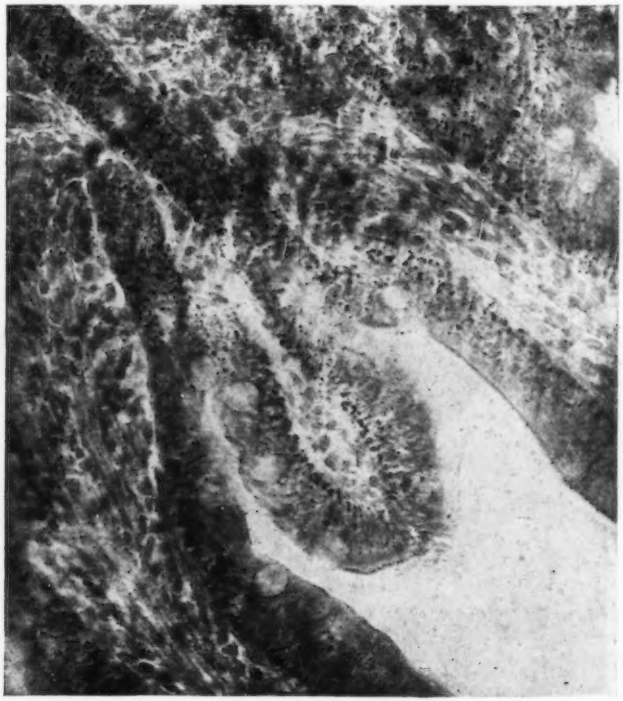

Fig. 3. A photomicrograph of a section of the duodenum of a cat showing droplets of fat in the bases of the villi and the upper portion of the intestinal glands. The cat was starved 28 hours and killed with chloroform. × about 293.

tendency to arrange themselves like a string of beads along the long axis of the cells. They do not appear in the outer portion of the cell facing the lumen of the stomach but occupy the basal two-thirds. The nuclear zone itself is comparatively free from them. Similar fat droplets are also present in the fundic or basal region of the gastric glands,

where they vary in quantity from large numbers to a doubtful presence. Figure 2 is a photomicrograph of a section from the stomach in which an unusually large amount of fat was demonstrated. Along the pits, neck and tubules of the gastric glands very few granules were seen and in a number of cases it was very doubtful if any were present. In the sections that showed the granules best they had a tendency to crowd about the base of the cells and appeared to be very similar to those in the border cells, except perhaps that their diameters were greater.

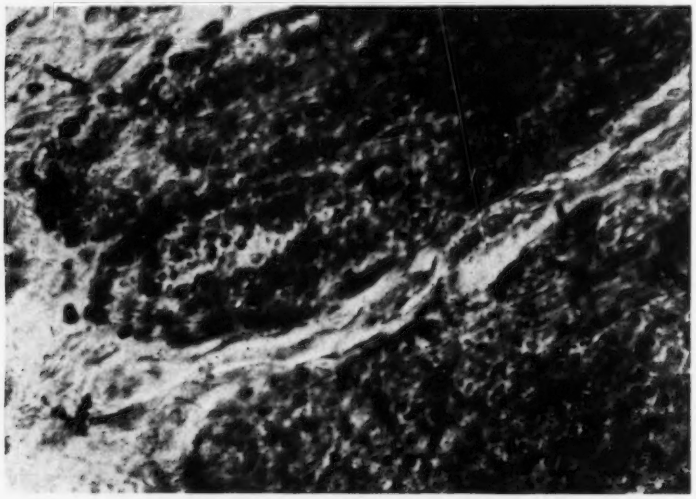

Fig. 4. A photomicrograph of a section of the duodenum of a cat showing droplets of fat in the depths of the intestinal glands. The cat was starved 27 hours and killed by a blow on the head. × about 293.

Obviously, I can agree with Greene and Skaer ('13) as to the presence of these droplets in the stomach mucosa and the gastric glands. The failure of Gay and Southard to demonstrate fat in the gastric mucosa of normal animals may have been due to the staining method employed, or it may be that the gastric mucosa of the guinea pig does not present a fat picture. Certainly there can be no doubt about the occurrence of fat droplets in the gastric mucosa of cats.

In all of the animals fat droplets were demonstrated also in sections from the duodenal wall. As in the gastric mucosa, the quantity

varied but the fat picture was at times striking. Usually the droplets were absent from the cells of the mucosa located along the free ends of the villi, while in those located at the base of the villi they were easily demonstrable. Along the intestinal glands the granules were very abundant and extended from the mouths of the glands into their

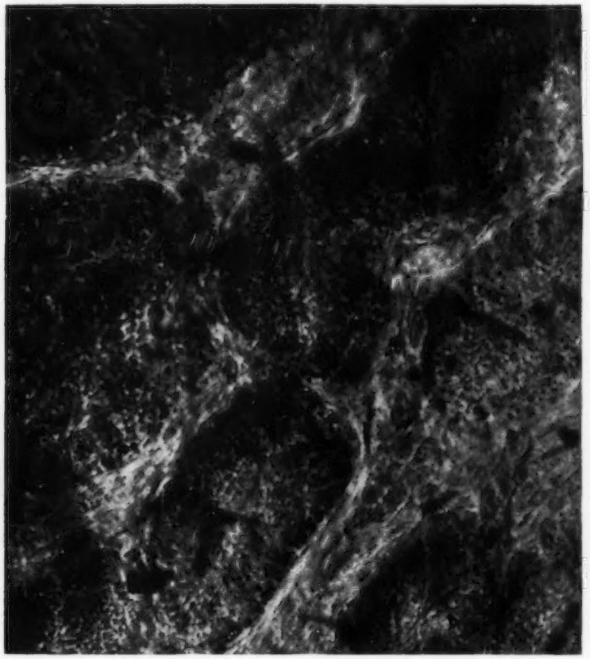

Fig. 5. A photomicrograph of a section of the duodenum of a cat showing droplets of fat in Brunner's glands. The cat was starved 27 hours and killed by a blow on the head. × about 293.

fundic region. Similarly, the granules were found in the duodenal or Brunner's gland. Here the amount varies greatly. In some cases the droplets could not be demonstrated while in others they appeared in great numbers, giving the glands a brilliant red color (fig. 5). At the base of the villi, in chosen sections that demonstrated them well, the fat droplets were located definitely at the basal portion of the cells (fig.

3). It was difficult to locate them accurately in the cells of the intestinal glands, on account of the thickness of the frozen sections and the swollen condition of the glands due to the starvation of the animal (figs. 3 and 4). In the duodenal glands of one of the animals the cells were crowded with these fat droplets, which were larger than the granules found elsewhere in the alimentary tract and any accurate description of their distribution was impossible. The quantity of fat in the cells at the base of the villi as well as in those of the intestinal glands was

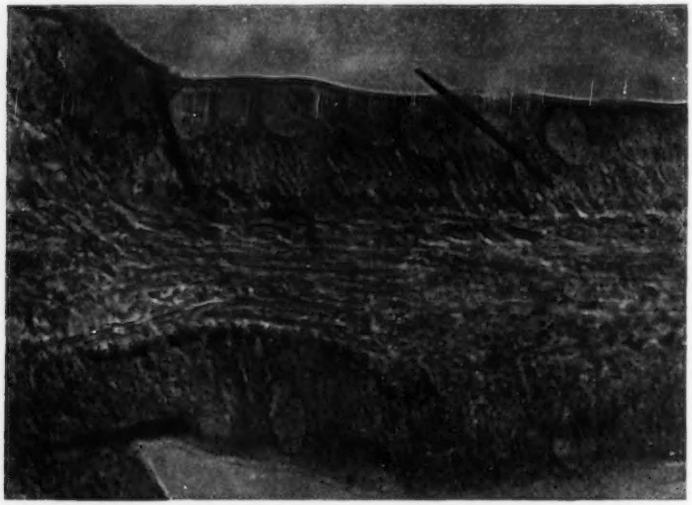

Fig. 6. A photomicrograph of a section of the small intestine of a cat showing droplets of fat in the lower portion of a villus. The cat was starved 28 hours and killed with chloroform. × about 293.

found to be approximately equal throughout sections from the same animal.

Fat droplets were present in all sections of the small intestine in every one of the animals examined, with the possible exception of one that had been killed by a blow on the head. In another that had met death in a similar manner there were present a large number of the granules. This is another example of the variability of the fat in these animals. In the lower part of the small intestines the granules were abundant along the tubules of the glands. As in the duodenum, the

droplets showed a tendency to disappear from the cells of the mucosa as the free ends of the villi were approached, but were very prominent at their base (fig. 6). Here, for the most part, they occupied the basal portion of the cells and toward the free margin were few in number. Due to the thickness of the sections, I was unable to decide definitely whether the droplets were confined solely to the simple columnar epithelial cells or were present in both these and the goblet cells. It is very difficult to describe their distribution in the tubules of the glands. They appear to be more or less evenly distributed but are not uniform in size. In general the picture here is very similar to that described for the duodenal area. In the stroma of the villi, beneath the mucosa, similar droplets may be present. In the stroma between the tubules of the intestinal glands there appeared to be very few.

In seeking an explanation for the presence of the fat droplets several possibilities are to be taken into consideration. The fat picture may be due to any one of the following causes: (1) To fat in process of absorption; (2) to the mobilization of fat; (3) to injury to the cells; (4) to technical errors (a pseudo-fat picture); (5) to the normal fat constituent of the cells.

1. Are we dealing with a fat picture which is the result of fat absorption? There are several counter-indications to this possibility: *a*, These animals were starved from 24 to 28 hours; *b*, there were negative indications of any fat-absorbing phenomenon in progress at the time of autopsy; *c*, it would be difficult to explain by this hypothesis the presence of fat droplets in Brunner's glands.

2. Regarding the mobilization of fat Greene and Skaer, '13, state:

> In the early stages of fasting the labile substances, namely, the carboydrates, are quickly used for the production of energy. A little later the fats of the adipose tissues and other more fixed substances are drawn upon. In this later stage the lipase-producing tissues are doubtless strongly stimulated to increased activity for the accomplishment of the transportation of fats. Lipase was proven by Lovenhart to be a normal content of a large number of tissues of which certain glandular tissues are particularly mentioned by him. To these tissues ought to be added the gastric glands which are lipase producers. If one assumes that an excessive production of lipase takes place in these glands at the time during fasting when the fats are being dissolved from the storage tissues, and are present in a relatively high per cent in the circulating fluids, it follows that there will be an increased synthesis of fat in the lipoid producing tissues themselves.

The presence of fat droplets in the cells may be accounted for thus in some cases but in the present studies, in view of the short period during which the animals were kept without food, it is quite improbable that

they had reached the stage of starvation at which fat is being mobilized, as outlined in the above quotation. They were starved 24 to 28 hours, probably not an uncommon occurrence for a cat.

3. A normal amount of fat may be brought to any cell but because of some injury to the cell itself this fat may not be consumed in the normal way and may collect within its borders. Such injuries may be toxic or circulatory. In this instance, however, such an explanation seems quite unsatisfactory. Considering the swiftness with which the animals were killed, it is highly improbable that we are dealing with impaired cellular consumption of fats. Furthermore, it is known that lipoids exist in invisible form within the cells. Injury to the cell may result in disintegrating these lipoids and bringing them into visible evidence. In this way a fat picture may be produced. The question arises, therefore, has any factor been introduced into these experiments that might result in the disintegration of the lipoids in the tissues? This point should be carefully considered, in view of the findings of Gay and Souhard ('07).

For a time it was believed that the fat picture might be accounted for by the assumption that the chloroform used to kill the animals had acted thus on the tissue. For this reason other animals were killed with gas and by blows on the head. Since these methods did not alter the picture to any marked degree, such an explanation would be faulty and questionable.

4. As stated before, in the staining method used in these investigations there resulted a red precipitation. The reason why no attempt was made to avoid this has already been explained. At first glance it may appear that these precipitated particles may have been mistaken for fat droplets. To safeguard against such an error a careful study was made of the precipitate, and moreover all observations were checked by examining fat-free sections with fat-containg sections.

5. It is possible to think of the cells as passing normally through a definite cycle of functional activity, at certain periods of which their lipoid constituents can be demonstrated by histological methods while at others this is not possible. This hypothesis would no doubt account in a more satisfactory manner for the foregoing observations than any of the other possibilities here considered. In those cases in which the fat content was abundantly demonstrated we were dealing, according to this reason, with the period of the cell's activity when the lipoid element was most susceptible for histological demonstration. Again, those cases in which very few or no granules were found represented a period

in the cycle during which the fat or lipoid elements were less susceptible. In this way the variability in the quantity of the fat in the different animals may be explained. Moreover, the presence of fat in such structures as Brunner's glands would in no way conflict with this hypothesis, and the observations of Greene and Skaer ('13) lend support to this suggestion. In their animals they found that the fat content in the stomach disappeared during the early stages of fat absorption. This observation would strongly indicate that these cells may actually possess such a cycle of activity.

SUMMARY

In this paper there has been outlined a histological method by which the fat content of the mucosa and adjoining glands may be satisfactorily demonstrated in moderately starved animals. The appearance of the fat content of these tissues—namely, the superficial epithelium of the stomach and gastric, duodenal and intestinal glands—has been described, due consideration being given to the size, shape, location and quantity of the droplets.

Of various hypotheses the following one has seemed to offer the most satisfactory explanation for the observations noted herein; i.e., that normally there is fat in the epithelial cells of the stomach and intestine which is not associated with the phenomenon of fat absorption; that this normal fat varies, however, with some definite cycle of functional activity of the cells themselves; that at certain periods the lipoids are in such a condition that they can be demonstrated by histological methods while at others they are not demonstrable. The normal presence of these lipoids must be taken into account in estimating experimental results.

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EXTIRPATION OF THE DUODENUM

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The effect of extirpation of the duodenum in the experimental animal has been in dispute since the early work of Minkowski and Pflüger. In 1889 von Mehring and Minkowski (1) demonstrated that complete removal of the pancreas in the dog produced a fatal diabetes. Pflüger (2) found that in the frog diabetes followed total removal of the pancreas but that also a more severe diabetes followed removal of the duodenum or section of the peritoneum between the pancreas and the duodenum. A great deal of experimental work was done on this duodenal-diabetes hypothesis before it was finally discarded.

Ehrmann (3) found that complete removal of the duodenum in the dog resulted in death in several days to a week, with acute pancreatitis and fat necrosis. René Lauwens (4) was able to keep a dog fourteen days after total extirpation of the duodenum. Rosenberg (5) reported five cases of duodenal extirpation in the dog, one of the dogs being still alive twenty-three days after the operation. Minkowski (6) kept a dog several weeks after removal of the duodenum and found only a temporary glycosuria. Bickel (7) also reported a dog living four and one-half weeks after removal of the duodenum. Pflüger (8) criticised the work of these men, claiming that in their experiments not all of the duodenum was removed. S. A. Matthews (9) found that extirpation of the first six inches of the duodenum was invariably fatal. The dogs displayed no serious symptoms for twenty-four to thirty-six hours but very shortly thereafter died. If a piece of the duodenal mucosa was transplanted into the intestine lower down and the remaining duodenum removed, the dogs lived. Death always followed subsequent removal of the transplant. Thus Matthews claimed that the duodenum is as necessary for life as the adrenals or parathyroids, probably through some hormone function other than that concerned with the elaboration

of secretin. A. P. Matthews (10) states that if experiments are made so that the duodenal juice (succus entericus) is drained to the exterior through a fistula, the animals die with apparently the symptoms of complete extirpation of the duodenum. He suggests that death may be due to the rapid excretion of some necessary substance through the duodenum to the exterior, or to the loss of some substance normally elaborated by the duodenum which is necessary to the function of the intestine lower down. From work on experimental intestinal obstruction Draper (11) has come to the conclusion that under certain pathological conditions (obstruction) the cells of the duodenal mucosa become perverted and secrete into the blood stream a powerful toxin. Whipple (12) and his collaborators, working on the same problem, have come to somewhat similar conclusions. Under conditions of obstruction the mucosa of the duodenum and possibly jejunum secretes a toxin of a proteose nature both into the lumen of the intestine and into the general circulation. The experimental results published by Draper and Whipple have led clinical men to attribute certain disorders in their patients to some disturbance in this function of the duodenum. Kanavel (13) suggests that death, in rupture of the duodenum, is due not to the ensuing peritonitis but to the absorption of some toxin from the duodenal mucous membrane. Bloodgood (14) thinks that death in some cases of duodenal dilatation likewise may be due to some disturbance of this internal secreting mechanism. He suggests the term "physiologic death."

In their experiments Minkowski, Pflüger and Bickel were intent mainly on severing all nervous connections between the pancreas and duodenum. Apparently they did not have in mind a specific internal secreting function of the duodenum and so may not have taken special care to remove all of the duodenal mucosa. According to S. A. Matthews, a small amount, a patch of mucosa 1.5 x 3 cm., suffices for life. Like Pflüger, Matthews holds that previous workers, reporting successful results, did not remove all of the duodenum.

The question of a specific internal secreting function of the duodenum, aside from the mechanism concerned with the elaboration of secretin, of such a nature as indicated by the work of Matthews, Draper and Whipple, is of great practical importance as well as biologic interest. We became interested in the question during the course of some work on the nature of the toxemia in intestinal obstruction and experiments of the following nature were performed.

Extirpation of varying lengths of the intestine. Removal of varying lengths of the jejunum and ileum produced no other effect than some nutritional disturbance similar to that described by Underhill (15) in experiments on dogs in which the small intestine was short circuited (functionally resected). The mucosa of the combined jejunum and ileum does not secrete or manufacture a necessary substance and animals can survive for months after the removal of the small intestine, the duodenum and colon remaining intact. All of the effects can be accounted for simply as due to the loss of an important digestive and absorptive organ.

Extirpation of the duodenum. Removal of the duodenum presents greater surgical difficulties. Because of the extreme vascularity of the duodenum, the intimate relation to the liver, stomach and pancreas many animals die from shock, internal hemorrhage or acute pancreatitis. There is always a profound disturbance of the functions of the liver, stomach and pancreas. The duodenum was removed from sixteen dogs and their behavior carefully observed. In each case the pyloric part of the stomach, the entire duodenum and the first part of the jejunum were removed. This of course necessitated a dissection of the ligament binding the lower duodenum and upper jejunum in order to permit the reëstablishment of intestinal continuity. In eleven of the dogs the operation was performed in two stages. At the first operation the pylorus was divided, both ends closed and an anterior gastro-enterostomy performed with the middle jejunum. After recovery from the first operation, a second was done and the duodenum removed as far as the gastro-enterostomy. The bile and pancreatic ducts were tied and the gall bladder drained, in some cases into the jejunum, in others to the exterior. Most of these dogs died in two or three days but one lived twelve days. At autopsy there was usually a general abdominal fat necrosis and it is our opinion that this, combined with the unavoidable injury to the pancreas in separating it from the duodenum, is the cause of death in these cases. The remainder of the dogs were operated in the following manner. The common bile duct was ligated and cut, the pylorus divided, the pancreas opposite the pylorus ligated and divided with a cautery and the entire duodenum with the adherent pancreas as well as the upper jejunum removed. Enough of the pancreas was left to prevent the onset of diabetes. As evidenced by the post-operative course of these animals and by subsequent post mortem examination, there was less danger of producing pancreatitis with this operation than when the pancreas was separated from the

duodenum. The middle jejunum was sutured to the divided pylorus and the gall bladder drained. The operation was completed at one stage and this proved to be a great advantage over the two-stage operation because of the extensive adhesions in the latter case. There was less gastric disturbance when the jejunum was sutured directly to the cut pylorus than when a gastro-enterostomy was done. All of the dogs were given glucose dissolved in Ringer's solution hypodermically daily for the first week. Most of these animals recovered from the immediate effects of the operation, displayed no untoward symptoms for five or six days but very shortly thereafter died. One of the dogs lived three weeks and one three months after the complete extirpation of the duodenum. Confirming the observations of Minkowski, these dogs showed only a temporary post-operative glycosuria and soon became sugar-free, remaining so till death.

The dog that survived three months showed, naturally, a very marked nutritional disturbance and it was only by careful feeding and attention that it was kept living for that period. There was a constant loss of body weight in spite of a liberal diet of lean meat and sugar. That there was an incomplete digestion of the food fed was evident from the almost constant appearance of undigested meat in the feces. It was soon apparent that the animal was starving in spite of efforts to maintain nutrition. Apparently the gastric juice alone, in the absence of bile, pancreatic and duodenal juice, does not suffice for the utilization of proteins. It is probable that the animal could have been preserved indefinitely in nitrogen equilibrium if a sufficient amount of protein in the form of peptones or amino acids had been supplied, along with sufficient carbohydrates for energy purposes. The symptoms produced in this dog by the complete extirpation of the duodenum are in all probability due simply to the disturbance in digestion. The duodenum or duodenal mucosa is not itself essential for life and is certainly not comparable to the adrenals or parathyroids in this respect. These experiments also establish the fact that the duodenum does not supply a substance which is necessary to the function of the intestine lower down. Intestinal motility was normal, or if anything exaggerated, after the removal of the duodenum.

The question of the toxicity of normal intestinal juice. Some workers (Draper) have concluded on the basis of animal experiments that the secretion of the duodenum and upper jejunum is toxic but that it is normally neutralized by the secretions of the intestinal mucosa lower down. One of us during the course of some experiments on the toxemia

of intestinal obstruction found that dogs could survive open isolated loops of the dodenum or jejunum in which the respective secretions are poured drectly into the abdominal cavity and absorbed. These dogs displayed no toxic symptoms. In a recent communication Davis and Stone have verified our contention that the succus entericus is not toxic when secreted. They found that the fresh secretion produced no untoward symptoms when injected intravenously in dogs.

The question of the toxicity of intestinal juice under pathological conditions. Whipple and his coworkers have concluded that under certain pathological conditions (obstruction, closed intestinal loops) the mucosa of the duodenum and jejunum assumes a perverted function and secretes a toxic proteose both into the lumen of the intestine and into the blood stream. When such pathological conditions are produced in the experimental animal there is no doubt that poisons accumulate in the obstructed intestine or in closed intestinal loops and that the dog shows symptoms of marked toxemia. In the experiments reported it seemed entirely possible that the toxic substances could be the products of bacterial activity. We know that exceedingly toxic substances such as the amines are produced in the intestine by the action of bacteria on certain amino acids. It seemed that the presence of bacteria should be definitely excluded before any conclusions were made regarding the toxicity of the secretion as it is formed by the duodenal cell or as it is found in the lumen of the intestine. When such precautions were taken—isolated intestinal loops rendered sterile by prolonged drainage into the abdominal cavity—it was found that many procedures, formation of closed loop, complete occlusion of the blood supply with resulting autolysis of the loop, which invariably caused severe toxemia and death when bacteria were not excluded, did not produce any noticeable symptoms.

Does the duodenum excrete some necessary substance in the duodenal juice? According to A. P. Matthews, if the duodenal juice is drained to the exterior through a fistula, death always results with apparently the same symptoms displayed by animals following a complete extirpation of the duodenum. The duodenum may excrete some necessary substance in the duodenal juice which is normally reabsorbed by the intestine lower down. A number of experiments were done to determine this point. If a fistula of the duodenum below the entrance of the bile and pancreatic ducts (fig. 1) be made the animals usually die within three days.

If however the operation be done as in figure 2, so that there is no blind stump of the first part of the duodenum, some of the animals live indefinitely and show no untoward symptoms whatever. The secretions of the duodenal mucosa below the entrance of the bile and pancreatic ducts do not contain any substance necessary to the organism.

If the secretions of the entire duodenum be drained by means of an abdominal fistula, the situation is similar to that following complete extirpation. The injury to the liver, pancreas and stomach as a result of

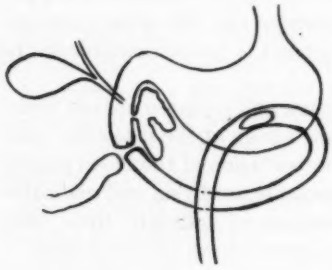

Fig. 1

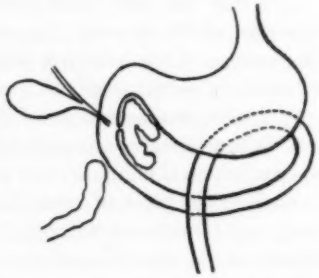

Fig. 2

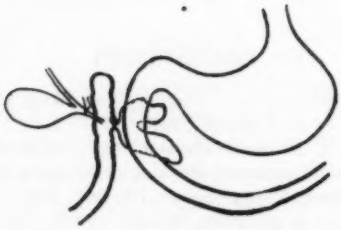

Fig. 3

the operation, together with the loss of three important digestive juices, presents such an abnormal situation that one need not postulate the loss of some necessary substance to explain the death that usually ensues. A number of dogs were operated as in figure 3. Most of the animals died within three days but two lived six days and one ten days. Dr. James J. Moorhead of Chicago was able to keep a dog twelve days after an operation similar to that described above. In addition to the disturbance in digestion produced by the deprivation of bile, pancreatic and duodenal juice, a slight occlusion of the fistulous opening in the skin produces a dilatation of the entire duodenum, which in itself is a

serious condition. For this reason this operation was not as successful as complete extirpation of the duodenum. There is no evidence, however, that the duodenal juice contains anything vitally necessary to the organism.

CONCLUSIONS

1. Animals can survive indefinitely a complete extirpation of the combined jejunum and ileum.

2. A dog was kept three months after a complete removal of the pyloric part of the stomach, the entire duodenum and the upper jejunum. The mucosa of this region of the digestive tract is not comparable to the adrenals or parathyroids in function.

3. The normal secretions of the duodenum and jejunum are not toxic.

4. When bacteria are excluded from the lumen of the intestine, various pathological changes even to complete occlusion of the blood supply to an isolated piece of intestine with resulting autolysis and reabsorption, can take place without the elaboration of sufficient toxic substances in the cells themselves or in their secretions to kill the animal.

5. The duodenum does not excrete in the duodenal juice any substance necesssary for life or for the function of the intestine lower down.

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THE VENO-PRESSOR MECHANISM

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This paper is the outcome of a suggestion by the Committee on Physiology of the National Research Council that the author investigate the question of a veno-pressor mechanism. It was believed that the derangement of such a mechanism might be a contributing factor in the stasis of blood which, in most instances at least, characterizes the condition of shock.

The relationship of the facts observed to shock has not been studied. It is not improbable that there is a relationship but it is not one easy of exact demonstration. The facts, however, are of interest in themselves and their bearing upon the larger problem, if there is such bearing, may be left to the future.

EXPERIMENTAL

Dogs were used and the experimental results are limited to observations on the sigmoidal area of the large intestine, approximately 8 cm. in length. The part receives its blood supply chiefly through a large branch of the inferior mesenteric artery and is drained by a good sized mesenteric vein. It is innervated by a nerve trunk originating in the inferior mesenteric ganglion which runs to the gut closely adherent to the artery. The technical procedure followed was to lay double mass ligatures about the gut and to cut between them above and below this area, to sever the intervening mesentery and to suspend the preparation by the ligatures about either end so that it hung free of pressure disturbances from the neighboring coils of intestine. The vein and artery were then cannulated and the blood washed out of all the vessels by arterial perfusion with warm Ringer's solution. In the earlier experiments this washing was followed by perfusion with Ringer's solution containing a suspension intended to produce capillary block. In the later experiments this step was omitted because of the lack of assurance that the block was in the capillaries and not in the arterioles

at a point such that contraction of these vessels might contribute to the rise of pressure in the vein. A further reason for its discontinuance was that the rise of pressure in the vein occurred whether the blocking suspension was introduced, whether the artery was simply clamped or whether the artery was left open. The final practice adopted then was to leave the artery open and it was assumed that if arterial contraction did occur it would tend to pass the contained fluid backward through the open artery rather than into the vein. The necessity for such an assumption is unfortunate; nevertheless, since the pressure on the arterial side was, by the procedure, at zero or even below zero (the open artery hanging down from the suspended gut) while the pressure on the venous side was 6.0 to 10.0 cm. of water, the likelihood of arterial constriction contributing to the rise of vein pressure is small. It is possible that arterial constriction by a peristaltic wave may sweep fluid onward into the capillaries and veins even when the arterial pressure is extremely low. But such activity is not demonstrable by the usual methods of studying vasomotor activity. In other words, vasoconstriction as usually produced is accompanied only by a fall in venous pressure. Under the experimental conditions, then, the observed rise of venous pressure whether due to constriction of arterioles, capillaries or veins would seem of necessity to be associated with a veno-pressor mechanism since the effect is to produce an active rise of venous pressure.

Attempt was made to produce a capillary block by perfusion through the vein instead of through the artery. This proved to be impossible, as has been noted by Mall, because of the valves. After the artery was relieved of the excess of perfusion pressure there was apparently little or no tendency for the fluid on the venous side to leak back, presumably because of valves, as indicated by the stability of the venous pressure.

After the blood was completely washed out of the vessels the vein was connected with a water manometer which served to record changes in venous tone. In most of the experiments in addition to the vein manometer a second manometer of the same bore was connected with the lumen of the intestine, a cannula having been included in one of the mass ligatures for this purpose, so as to follow concomitant changes of pressure due to contraction of the intestinal musculature.

The pumping action of the intestinal villi observed by Hambleton (1) as a possible factor in the venous blood pressure changes noted in these experiments has not been considered. It is conceivable that such a factor is involved but no means are at present available for its investigation.

THE PERIPHERAL MECHANISM

With the preparation as above described, it is an easy matter to demonstrate the peripheral veno-pressor mechanism. Section and peripheral stimulation of the nerve trunk running from the inferior mesenteric ganglion to the part under investigation almost invariably gives a distinct rise of pressure in the vein as read on the water manometer. This result is as easily obtained if the preparation is entirely removed from the body and the reaction may be obtained for an astonishingly long time. In an experiment directed particularly to this point a good reaction was shown upon faradic stimulation of the nerve an hour and fifteen minutes after the circulation was isolated.

In a series of experiments notes of thirty-one observations were preserved showing a rise of vein pressure independent of pressure changes within the lumen of the gut. There is usually a latent period of some seconds after which the rise of pressure sets in. The rise is slow and steady and if the stimulus be of short duration may reach its maximum some time after the irritation has been removed. On the other hand a protracted stimulation results in a sustained elevation of the pressure. By the end of the second minute of such stimulation the preparation shows signs of fatigue and the vein pressure begins to fall. It was not determined whether this fatigue is localized in the nerve fiber, in the vein or in both.

In this series of thirty-one observations the rise of vein pressure in centimeters of water was as follows: 2.0, 2.0, 3.25, 0.75, 1.0, 7.25, 4.5, 4.5, 2.0, 2.5, 1.0, 0.75, 1.25, 0.75, 1.25, 0.75, 1.0, 1.5, 7.0, 5.0, 2.75, 2.25, 0.75, 1.75, 1.5, 1.0, 2.0, 1.75, 0.75, 4.5, 5.5, 1.75 and 1.5 cm. These figures show a minimum rise of 0.75 cm. and a maximum rise of 7.25 cm. Within limits the strength of the stimulus determines the extent of the rise of pressure but the chief factor appeared to be the condition of irritability of the preparation for at times the strongest stimulation fails to elicit any response and the greatest rise is not necessarily associated with the strongest stimulus. After the maximum has been reached the pressure falls back slowly so that the curve resembles a curve of the contraction of smooth muscle.

THE CENTRAL MECHANISM

The part played by the central portion of the veno-pressor mechanism is much less easy of demonstration. This was studied by leaving the nervous connection to the part intact. Time and again when the

peripheral mechanism gave a ready response to direct nerve stimulation no response was elicited through the central mechanism so that the conviction was firmly established that the nerve centers controlling the regulation of venous tone are prone to lose their functional power under the abnormal conditions established by the experimental procedure. The operative exposure of the abdominal viscera and manipulations incident to the procedure subjected the anesthetized animal to extreme sensory stimulation adequate, it may be assumed, to start the train of events which ultimately leads to the condition of shock.

Under the most favorable experimental conditions a reflex rise of venous pressure was demonstrable but it could not be demonstrated repeatedly and was never demonstrated late in an experiment. But so far as the actual demonstration of the existence of a central veno-motor mechanism goes and aside from its possible significance in shock, we are concerned wth the positive evidence of central regulation.

The line of attack in these experiments was twofold: to study the effect upon the venous pressure of nerve section or of section of the spinal cord (pithing) and of central stimulation produced by sensory nerve irritation or by asphyxia.

Fall of venous pressure incident to nerve section. The simplest method of determining the presence of central venous tone was to cut the nerve and note the consequent fall in venous pressure. This procedure was instituted only when the vein manometer gave a constant reading. In seven experiments the fall in venous pressure was noted together with the time required for the manometer to again give a constant reading. The results were as follows: 2 minutes, 1.0 cm.; 4 minutes, 0.5 cm.; ½ minute, 0.5 cm.; 19 minutes, 4.0 cm.; 9 minutes, 4.0 cm.; ½ minute, 0.5 cm. and 2 minutes, 0.5 cm.

Fall of venous pressure incident to pithing. The animal was pithed by passing a small scalpel through the tissues at the base of the skull and transecting the cord at the foramen magnum. The effect of this procedure was usually to cause a transitory rise of venous pressure followed by a slow fall, the latter being like that observed upon nerve section. The experiments on cord transection, as above described, three in number, served to locate the central veno-pressor mechanism in the medulla. The results were as follows:

1. After 15 minutes pressure fell 4.0 cm.
2. After 1 minute pressure rose 1.5 cm.
   After 10 minutes pressure fell 3.25 cm. below original level.
3. After ½ minute pressure rose 1.0 cm.
   After 9 minutes pressure fell 0.5 cm. below original level.

Not much emphasis is to be laid upon these results or upon the results of nerve section. They indicate an influence of a medullary center upon the venous tone but the evidence is not so striking as might be wished because of the slowness with which the change is brought about. Possibly there is little central tone under the experimental conditions or possibly the load-tension on the vascular muscle was insufficient to cause prompt relaxation. In any case the results are much less convincing than those which exhibit a rise in pressure upon sensory stimulation.

Rise of venous pressure incident to faradic stimulus of saphenous nerve. The saphenous was chosen as a convenient sensory nerve for the study of reflex effects upon the venous blood pressure and all the results reported were obtained by its stimulation. In eight experiments twenty-two observations were made which gave a rise of venous pressure. In these the rise of vein pressure in centimeters of water was as follows: 1.0, 0.5, 0.5, 0.75, 0.5, 2.0, 1.25, 1.5, 1.5, 2.0, 3.5, 2.0, 0.5, 1.5, 1.5, 1.0, 0.5, 1.0, 0.5, 1.0, 0.5 and 0.75 cm. These figures show a minimum rise of 0.5 cm. and a maximum rise of 3.5 cm. In the case of the peripheral mechanism the chief factor controlling the response appeared to be the irritability of the preparation. So here, in the case of the central mechanism, the irritability of the veno-presso center rather than the strength of stimulation was the controlling factor.

It will be noted that the rise of pressure produced reflexly by sensory nerve stimulation is generally less than the rise produced by direct stimulation of the veno-motor nerve fibers themselves—the maximum rise for the latter being 7.5 cm. as compared with 3.5 cm. for the former. This was not unexpected since the mediation of the central mechanism would predicate a lesser response. It should perhaps be stated that these results were wholly independent of pressure changes within the lumen of the gut and of pressure from neighboring viscera.

Rise of venous pressure incident to asphyxiation. Occlusion of the trachea proved to be the most satisfactory way of producing a rise of pressure by the activation of the veno-pressor center. Not infrequently when sensory nerve stimulation was without effect a considerable rise in vein pressure was produced by asphyxiation and the inference is made that asphyxia is the more potent stimulus. In eight experiments eleven observations were made. In these the rise of venous pressure in centimeters of water was as follows: 5.0, 2.75, 1.25, 2.5, 1.5, 2.25, 4.5, 1.5, 4.0, 3.25 and 2.0 cm. The minimum rise was 1.25 cm. and the maximum rise was 4.5 cm. In several experiments a pre-

liminary slight fall in pressure was noted which must have been produced by a transitory loss in central tone. This fall, however, shortly gave place to a rise in pressure which sometimes progressed beyond the period of occlusion of the trachea. In two experiments the manometer readings were strikingly confirmed by observation of the intact loops of intestine lying near the preparation. In the beginning the larger veins particularly along the mesenteric border were distended and conspicuous. As asphyxia progressed these veins decreased in size and became relatively inconspicuous. Along with this change in the appearance of the veins the intestine became blanched. This change in the appearance of the veins can not be explained by an asphyxial constriction of the arterioles because this would not empty the veins and in the present case the writhing movements which accompany asphyxia were unusually slight and did not appear until after the change described in the veins had developed. A similar change in the veins in the part connected with the manometer was not apparent because these veins had been washed free of blood and were therefore relatively inconspicuous. The veins which showed this reaction were, of course, filled with asphyxial blood and it is possible to explain the constriction on Henderson's hypothesis (2) of the chemical veno-constrictor effect of carbon dioxide. The writer is not, however, impressed by this hypothesis especially because of repeated confirmation of Bayliss' earlier observation that carbon dioxide relaxes vascular muscle (3). In view of the results reported in this paper it seems very probable that the constriction noted in the veins was associated with the central (nervous) effect of the asphyxia.

In the asphyxia experiments as well as in the experiments dealing with saphenous stimulation care was necessary lest the excessive respiratory movements result in compression of the preparation in such a way as to raise passively the vein pressure. To obtain assurance that such a passive factor was not in force unknown to the observer an experiment was performed in which after obtaining an asphyxial rise of vein pressure in the usual manner the nerve to the part was cut and asphyxia again instituted. With the nerve cut an extreme asphyxia was entirely without effect upon the vein pressure.

INTESTINAL MECHANISM

Under this heading are considered observations in which active changes in the intestinal musculature obviously contributed or at least occurred coincidently with considerable alterations of the vein pressure. The

data at hand are quite limited and no effort was made to study the condition carefully. In the cases in which it occurred the vagi were intact and ether alone was used for anesthesia. It would seem that under certain conditions intestinal movements may materially influence the movement of blood in the portal system as suggested by Mall. At times the pressure within the intestinal loop remains unchanged during a rise of vein pressure, at times it shows a decided fall and at times again it may show a decided rise. To give an example which shows the coincident rise of both loop and vein pressures it was found that with vagi intact and with ether alone as anesthesia, stimulation of the saphenous nerve and asphyxia gave the following pressure changes:

	VEIN PRESSURE ROSE	GUT PRESSURE ROSE
	cm.	*cm.*
First saphenous stimulation	7.0	24.0
Second saphenous stimulation	3.5	8.5
Third saphenous stimulation	1.5	17.0
Occlusion of trachea	5.25	25.0

It is clear that in a case such as this the rise of vein pressure is probably in large part at least passive. This function of intestinal movement may well be of significance under normal conditions although it is not to be regarded as essential to the veno-pressor mechanism.

The reader will appreciate that the results given above represent an extension of the observations made by Mall in 1896 (4). Mall showed that splanchnic stimulation causes constriction of the portal vein and its tributaries. Recently Burton-Opitz (5) has confirmed these observations by another method. Mall, then, demonstrated the existence of a peripheral veno-pressor mechanism and it was a natural inference that such a mechanism should be under central and therefore reflex control.

Recent contributions to the shock problem (6) emphasize a chemical factor as determining the failure of the circulation. According to this view some toxic metabolic product dilates the capillaries and venules producing a peripheral stasis of blood. That a nervous control is not to be excluded is indicated by the experiments of Cotton, Slade and Lewis (7) which show that these small vessels respond to stimulation as was shown by Stricker in 1865 (8). It is possible that both nervous

and chemical factors may contribute to vascular relaxation in the capillary region in shock. If so, we should expect the more promptly acting nervous mechanism to instigate the changes which are subsequently accentuated by the processes due to chemical action.

SUMMARY

1. A preparation of the large intestine in the dog is described, on which a rise of venous pressure may be demonstrated independently of arterial and other factors.

2. The rise of venous pressure may be obtained by *a*, stimulation of the nerve to the part (peripheral mechanism); *b*, stimulation of a sensory nerve (central reflex mechanism); and *c*, central stimulation by asphyxia.

3. Attention is called to the fact that activity of the intestinal muscle may contribute to the movement and pressure of blood in the portal venous system.

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